

THE RELATIONSHIP BETWEEN AUXIN
PRODUCTION CAPACITY BY RHIZOBACTERIA
AND WHEAT BIOMASS

By

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Abstract: The plant hormone auxin is known to be involved in growth responses in plants. Bacteria also produce auxin for some unknown reason. In this work we seek to better understand the role of auxin produced by rhizobacteria on plant growth. We determined the relationship between auxin production capacity and wheat biomass for both the rhizosphere and endorhizosphere inhabiting bacteria. A total of 96 wheat plants were grown in two different soil types namely, Teller fine sandy loam and Easpur loam. From 96 plants, 20 were randomly selected from each soil type. Plants were harvested and the shoot biomass determined. A total of 4320 individual bacteria were isolated between the two soil types. Isolates were dilution plated, randomly selected and categorized after 4 days of growth as large (≥ 2 mm) and small (< 2 mm) colonies. Selected isolates were grown for 4 days in 1x TSA, cultures centrifuged and the supernatants transferred to the 96 well plate for the auxin assay based on the Salkowski assay. Auxin concentration in each culture supernatant was measured using a spectrophotometer at 540 nm. In a separate experiment using 576 randomly selected rhizobacteria auxin production capacity and cell growth in culture was measured over a 4 day period at 540 nm and 595 nm spectrophotometrically, respectively. There was a high correlation ($R^2 = 0.94$) between cell growth and auxin production capacity in both large and small growing colonies indicating a strong relationship between bacteria cell growth and auxin production. Examining the correlation between auxin production capacity and biomass productivity from the 20 selected plants was determined by least square regression. The relationship was in most cases negative and non-significant, except for large colony bacteria in the endorhizosphere of the Teller fine sandy loam soils, which was negative and significant (p value ≤ 0.0148). Large colonies from both soils produced (33%) significantly higher average auxin concentrations (p value ≤ 0.005) than small colonies. There was no significant difference in auxin production capacity between the two soil types (p value ≤ 0.183). The result suggests that auxin production capacity by bacteria is not related to plant growth promotion.

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CHAPTER I

LITERATURE REVIEW

Wheat Production: The population around the world is drastically increasing with a current standing at 7 billion (Taylor & Koo, 2015). According to the global population estimates, population growth rate is expected to reach between 8.3 and 10.9 billion by 2050 (Taylor & Koo, 2015). Thus, in order to sustain this growing population, food production has to significantly increase. Presently, fertilizers and pesticide inputs are used to increase productivity and prevent pests and diseases. However, the use of these inputs continue to have dramatic effects on the environment. Hence, increasing food production requires the development of sustainable production systems with less or no environmental impact.

Wheat is a cereal grain that originated from the Levant region of the Middle East. It is the third most important cereal crop after rice and maize (Taylor & Koo, 2015). The annual wheat production in the world is approximately 713 million metric tons, which is grown on approximately 215 million hectares (Taylor & Koo, 2015). The major wheat producers around the world are the European Union, China, India, United States and Russia (Balkovič et al., 2014). The total amount of wheat produced by the US in 2013 was approximately 60 million tons (Balkovič et al., 2014). Wheat is a major source of caloric sustenance to a large part of world population. Next to rice, wheat provides more caloric.

than any other crop and is a good source of high quality protein, vitamins and dietary fiber. With rice, wheat is the world's most favored food staple. Wheat is process into flour to make bread, crackers, biscuits, pancakes, pies, cookies, muffins, rolls, and doughnuts etc. attesting to its culinary versatility.

The Plant Hormone Auxin: The discovery of auxin was first observed by Charles Darwin more than 100 years ago. Darwin noticed a bending on grass seedlings towards the sunlight, but when the plants were covered with foil, they no longer bended. Thus, Darwin hypothesized that, some plant growth changes are regulated 'by a matter which transmits its effects from one part of the plant to another (Darwin, 1888). These signaling molecules are transported from the side facing the light to the shaded area which stimulate a greater growth on the shaded side (Allan, 1977). Many decades after Darwin's proposed the existence of this plant hormone, one of the type of auxin chemically identified was indole-3-acetic acid (IAA) (Went & Thimann, 1937). Auxin was the first major plant hormone to be identified. The word auxin is generated from a Greek word (auxein) meaning to grow (Went & Thimann, 1937).

Auxin Functions: Auxin is referred to as a phytohormone that is associated with plant growth and development. In plants, growth is defined as a permanent increase in size and is caused by the growth of individual cells induced by hydrostatic expansion (Teale, Paponov, & Palme, 2006). Shoots grow from terminal buds and auxiliary buds located on the tips of the stem and in the axil of developing leaves, respectively. These buds are major sources of auxin production especially the terminal buds and act to coordinate cell growth processes. Auxin is also involved in the development of lateral root initiation through

multiple auxin signaling molecules (Lavenus et al., 2013). Formation of lateral roots is a significant mechanism that plant uses to increase their absorptive area. Lateral root development occurs as a result of division of selected root pericycle cells, which are adjacent to the protoxylem poles of the parent root (Beeckman, Burssens, & Inzé, 2001). Elevated auxin level causes the division of pericycle cells and lateral root initiation (Dubrovsky et al., 2008). Also, Casimiro et al., 2001 demonstrated that the movement of auxin through the root tip is vital in lateral root initiation. When Plant growth promoting rhizobacteria are inoculated in a plant, increased the numbers of root hairs and lateral roots, and at the same time shorten the shoot length (Spaepen & Vanderleyden, 2011). Thus, this resulted in a larger root surface area, and presumably an increase in mineral uptake from the soil (Spaepen & Vanderleyden, 2011).

Auxin is also involved in the transformation of root morphology. An increase in auxin level in the root of the plant stimulated the de-differentiation of pericycle cells (Karas & McCully, 1973) and multiple cell division in the root primordia, resulting in an increase in overall root size (MacIsaac, Sawhney, & Pohorecky, 1989). Arroo et al., 1995 demonstrated the effect of exogenous auxin applied in liquid grown cultures *in vivo*. Exogenous auxin was applied to a set of cultures and another set was left untreated. Auxin initiated high numbers of root primordia and within two days, they developed into lateral root with highly branched morphology. This increased growth rate, while cultures not treated with auxin had fewer root primordia and less growth rate.

The movement of plant towards stimuli is called tropism. Plants can adapt to various environmental stimuli such as gravity and light, by adjusting their growth

mechanism. The movement of the plant towards gravity is referred to as gravitropism. Charles Darwin was the first to report that roots showed positive gravitropism through their downward growth as a result of gravitational pull, while stems show negative gravitropism due to upward directional growth as a result of auxin redistribution (Darwin, 1888). The majority of the downstream gravitropism responses are auxin-dependent. Auxin plays an important role in the movement of the plant root towards gravity due to polar auxin transport (Swarup et al., 2005) by linking gravity sensing cells to the response cells, through the efflux and influx of auxin in the root (Haub, Gribble, & Jacobsen, 2011). The gravitropic effect is sensed in the collumella cells (root cap) where dense starch filled amyloplast or statoliths are deposited at the base of the root, causing it to move towards gravity (Sato, Hijazi, Bennett, Vissenberg, & Swarup, 2014) (Morita & Tasaka, 2004). Auxin transported through the root axis is responsible for the increased accumulation of statoliths at the basal part of the root (Peer, Blakeslee, Yang, & Murphy, 2011). Elevated auxin in the root inhibits growth on its lower side causing the root to bend towards gravity (Swarup et al., 2005).

The movement of the plant towards sunlight is known as phototropism. Darwin and his son observed etiolated grass seedling moving towards sunlight from a specific direction and concluded that auxin may be responsible (Darwin, 1888).

Auxin is also associated with the interaction between the shoot/root ratios. According to (Jiang et al., 2015), exogenous IAA applied to rice expressed a response gene which inhibited the development of the shoot apical meristem while it increased the size of the root apical meristem. Maitra & Sen, 1987 reported that some signal presumably from

the sink organs were responsible for the profound metabolic changes in the leaves (source) indicating a role in source sink relationship. Pathogens such as tobacco mosaic virus, rust, smut, powdery mildew, and *Pseudomonas* strains, have been proven to alter source-sink relationships to form efficient sink for photosynthate in areas of infection in wheat, maize, tomato and tobacco and in the model plant arabidopsis (Wright, Baldwin, Shephard, & Scholes, 1995) (Chou, Bundock, Rolfe, & Scholes, 2000; Herbers et al., 2000) (Doehlemann et al., 2008; Scharte, SchÖN, & Weis, 2005). (Depuydt et al., 2009). The conversion of source into a sink has been reported for different types of plant microbe interactions (Wright et al., 1995) (Chou et al., 2000) (Herbers et al., 2000) (Scharte et al., 2005) (Doehlemann et al., 2008). The transition from source to sink occurs simultaneously with changes in photosynthetic capacity (Chou et al., 2000). Transitions from source to sink in infected tissues occurs through the activation of invertases, which eventually leads to a buildup in carbon source levels which ultimately creates the establishment of a nutritious microbial habitat for the infectious bacteria (Depuydt et al., 2009). According to (Stes, Vandeputte, El Jaziri, Holsters, & Vereecke, 2011) when plants are infected with pathogens, the shoot remain immature and never transitions from source tissues to sink resulting into a convenient niche for both epiphytic and endophytic *Rhodococcus faciens* (Stes et al., 2011). Auxin is critical to the morphological development of the plant, especially in the patters of shoot branching (Domagalska & Leyser, 2011).

Pathways: Tryptophan has been identified as the main precursor for IAA biosynthesis pathways in bacteria (Spaepen, Vanderleyden, & Remans, 2007). Five different biosynthesis pathways have been identified among plant associated bacteria. In bacteria, the idole-3-acetamide (IAM) pathway is the best characterized pathway in which

tryptophan is converted to IAM by the enzyme tryptophan-2-monooxygenase (IaaM). Then IAM is converted to IAA by an enzyme IAM hydrolase (IaaH). The genes responsible for the conversion of IAM into auxin have been cloned from bacteria such as *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Pantoea anglomerans*, *Rhizobium sp.* and *Bradyrhizobium sp.* (Theunis, Kobayashi, Broughton, & Prinsen, 2004). *Erwinia chrysanthemi* is another bacteria known to produce IAA through the IAM pathway (Yang et al., 2007).

The next pathway is the indole-3-pyruvate (IPyA), which is the major pathway in plant IAA biosynthesis. The formation of IAA is through the conversion of tryptophan to IPyA which is then decarboxylated to form indole-3-acetaldehyde (IAAId). Finally, IAAId is oxidized to form IAA. The genes responsible for this process have been isolated from different bacteria such as *Azospirillum brasilense*, *E. cloacae*, *Pseudomonas putida* and *Pa. anglomerans* (Patten & Glick, 2002).

The tryptamine pathway is also used to synthesize IAA through the conversion of tryptophan to tryptamine and finally to IAA. This pathway has been identified in *Bacillus cereus* (Perley & Stowe, 1966). The tryptophan side-chain oxidase (TSO) pathway is also used to synthesize IAA through the conversion of tryptophan to IAAId which can be oxidized to form auxin. It has been demonstrated in *Pseudomonas fluorescens*.

Another pathway is the indole-3-acetonitrile (IAN), in which tryptophan is converted to IAN which is then oxidized to form IAA. This pathway has been found in bacteria such as; *Alcaligenes faecalis*, *Ag tumefaciens* and *Rhizobium spp.* (Kobayashi, Suzuki, Fujita, Masuda, & Shimizu, 1995).

Bacteria Produces Auxin: Interestingly, microorganisms also produces auxin in the form of indole-3-acetic acid (IAA) (Patten & Glick, 1996), which may perform a vital role in the growth and development of the plant (Khalid, Arshad, & Zahir, 2004). The production of this phytohormone by free living cultures is a great achievement for many phytopathogenic gall forming bacteria such as *P. anglomerans*, *P. savastanoi pv. savastanoi*, *P. syringae pv. syringae*, *Ralstonia solanacearum* and *Rhodococcus faciens* (Valls, Genin, & Boucher, 2006). Bacteria such as *Agrobacterium spp.* and *Pseudomonas savastanoi pv. Savastanoi* have been documented to produce auxin (Mole, Baltrus, Dangel, & Grant, 2007). *A. brasilense*, one of the most studied of the plant growth promoting bacteria synthesize IAA using the IPA pathway and the highest production is during the stationary phase (Spaepen & Vanderleyden, 2011). *A. tumefaciens* was known to encode genes responsible for the production of auxin which is found in the T-DNA of the bacteria (Spaepen & Vanderleyden, 2011) *Xanthomonas axonopodis* were found to produce high amount of IAA by constitutive gene expression and when exposed to a leaf extract from a host plant *Citrus sinensis* (Costacurta, Mazzafera, & Rosato, 1998). In *P. agglomerans*, IAA production increased when the bacteria was grown on plant leaf surfaces (Brandl & Lindow, 1997). The rhizobium sp. can induce the production of IAA using transcriptional regulators mechanism (Theunis et al., 2004). Many growth promoting bacteria produce auxin which is thought to assist in their plant colonization mechanism, including circumvention and phytostimulation of basal plant defense mechanism (Spaepen et al., 2007).

The Microbial Community: The microbial community has an essential role in the growth and development plants. These microbes boost the available nutrient (Dakora & Phillips,

2002), enhance the structure of the soil morphology (Amellal, Burtin, Bartoli, & Heulin, 1998), protect against pathogen (Mendes, Garbeva, & Raaijmakers, 2013) and provide substances (such as auxin) needed for plant growth (Dodd, Zinovkina, Safronova, & Belimov, 2010). The microbial community is so important to the plant that the plant provides approximately 21% of its net photosynthetic product to sustain the microbial community (Bisseling, Dangl, & Schulze-Lefert, 2009). The exact role of auxin production in the bacterial community is not known but is the subject of research today.

Auxin as a Signaling Molecule: Bacteria auxin can also be used as a signaling molecule which can have a direct effect on the bacteria community and bacteria physiology (Spaepen et al., 2007). Bacteria utilizes signaling molecules as a mechanism to ensure their adaptation and survival in the environment (Waters & Bassler, 2005). Indole is one of the most used signaling molecule by microbes (Lee & Lee, 2010). In *Escherichia coli*, indole is well known as a signaling molecule (Lee & Lee, 2010) and the cells associated with IAA are more resistant to various stress agent. IAA as a signaling molecule has been demonstrated in various bacteria such as the *A. tumefaciens*, in which IAA inhibited vir gene expression upon increased production of IAA by transformed plant cells (Liu & Nester, 2006). Also, in *Ps. syringae pv. syringae*, IAA was reported to be involved in the expression of syringomycin synthesis which is needed for complete virulence of the strains on stone fruit (G.-W. Xu & Gross, 1988). IAA as a signaling molecule was also demonstrated using *E. coli*, where IAA activated genes related to survival under adverse conditions which regulated the behavior of the bacteria and induced resistance to stress (C. Bianco et al., 2006). IAA synthesis was also observed in *Ralstonia solanacearum* (Valls et al., 2006) under adverse stress conditions (C. Bianco et al., 2006).

Negative Effect of Bacterial Auxin: The plant microbe interaction can be either mutualistic or pathogenic depending on the biological needs of the bacteria (Yue, Hu, & Huang, 2014). Bacterial produced auxin can have a positive effect or a negative effect on the plant. The effect of bacterial auxin in plant is contingent upon the amount of IAA produced and the sensitivity the plant tissues to changes in IAA concentration (Spaepen et al., 2007). Liu & Nester, 2006 reported that the growth of many plant associated bacteria were inhibited with high concentration of IAA ($200 \mu\text{l ml}^{-1}$), but soil bacteria were not inhibited indicating that the effect was location specific. Al-idani, 2011 reported a negative correlation between plant biomass and *in vitro* auxin production capacity. This was done by extracting isolates from plant categorized into high medium and low biomass. The high auxin producing bacteria were mostly associated with the low biomass plant while the low auxin producing bacteria were mostly associated with the high biomass plant. Sarwar & Kremer, 1995, also reported the negative effect of bacteria produced auxin. High amount of auxin produced by deleterious bacteria in the rhizosphere led to a significant reduction in weed seedling biomass. Deleterious rhizobacteria had been proven to cause reduction in seed germination and seedling vigor in weed seedlings (Kremer, Begonia, Stanley, & Lanham, 1990). The isolate (*Enterobacter taylorae*) with a very high auxin production capacity of $72.2 \mu\text{g ml}^{-1}$ was used to inoculate field bindweed resulting in a significant reduction in root length (Sarwar & Kremer, 1995). *Enterobacter taylorae* was also inoculated on wheat plant, and it also showed a significant decrease in the root length (Sarwar & Kremer, 1995). Nakbanpote et al., 2013, demonstrated the negative effect of bacteria produced auxin on rice seedlings. They isolated three bacteria from a saline soil contaminated with Zinc and cadmium, all of which were capable of producing IAA. These

isolates were induced in rice seedlings which resulted a significant decrease in the germination rate of the rice seedlings. The reduction in growth presumably occurred due to high auxin production by the isolates. Also, (Schroth, 1986) accounted that two strains classified in the family of *Enterobacteriaceae* reduced root elongation in sugar beet presumably as a result of the high IAA. Also, Hussain & Hasnain, 2011 studied the role of IAA in phytostimulation by rhizobacteria. Rhizobacteria from three different genera including; (*Pseudomonas*, *Bacillus*, and *Azospirillum*) were isolated and screened for IAA effect on plant growth in the field, resulting in reduced root length and overall wheat productivity. The range of auxin production by these rhizobacteria was from 0.02 to 10 µg /ml screening for IAA based on the Salkowski reagent (Hussain & Hasnain, 2011).

Positive Effect of Bacterial Auxin: Plant growth promoting rhizobacteria (PGPR) have been used to as inoculant to improve the growth and development of the plant. Ravari & Heidarzadeh, 2014 demonstrated the positive effect of auxin by isolating bacillus strains from wheat and tomato plant rhizosphere. The isolated bacillus strains where known to produce IAA, which were investigated for effectiveness on growth and yield under controlled environment. Two bacillus strains namely WHIr-15 and WHIr-12 produced maximum amount of auxin (16.2 and 14 µl ml⁻¹ respectively). *Bacillus* auxin producers had a positive impacts on wheat plant through the significant increase on the root length, root weight, panicle weight and increases wheat growth as compared to the control wheat.

Khalid et al., 2004 also reported the positive effect of bacteria producing auxin through the screening of effective PGPR strains for auxin production, plant growth and development under gnotobiotic conditions. A large number of rhizobacteria where isolated

from the rhizosphere soil of the wheat plants grown at different sites. These isolates were grown in tryptic soy agar medium and were selected for auxin production. These isolates produced auxin ranging from 1.1 to 12.1 $\mu\text{g ml}^{-1}$ without the tryptophan and with the addition of tryptophan the auxin production was significantly increased ranging from 1.8 to 24.8 $\mu\text{g ml}^{-1}$. The inoculated plants demonstrated an increase in root elongation, root dry weight, shoot elongation, and shoot dry weight. Thus, it was concluded that the strain with the highest auxin produced caused maximum increase in growth and yield of wheat.

Egamberdieva, 2008 demonstrated the positive effect of bacteria isolated from the rhizosphere and the phyllosphere of both wheat and pea plant. The bacterial strains were identified as *Pseudomonas*, *Bacillus*, *Kocuria*, *Microbacterium*, and *Cellulomonas* species. However, these isolates were found to produce indole-3-acetic acid (IAA) ranging from 2.0 μl to 2.70 $\mu\text{g ml}^{-1}$ (Egamberdieva, 2008). These isolate were inoculated in wheat plant and significantly increased the root, shoot, and dry weight of the plant. Lateral root were also significantly increased after inoculation with these bacteria. A *Sinorhizobium meliloti* was inoculated in *Medicago truncatula* which induced an increase in plant growth and improved resistance to salt stress presumably as a result of the bacteria's ability to produce auxin (Carmen Bianco & Defez, 2009). A study carried out by L. Xu, Xu, Jiang, Hu, & Li, 2015 reported that IAA producing bacteria were inoculated into peanut plants, which significantly affected the plant growth, plant nutrient concentration, soil nutrient concentration, soil microorganism and soil auxin concentration. The addition of the bacterium *Baccillus megaterium* significantly increased plant growth, plant height and shoot dry weight. The plant nutrient concentration and soil nutrient concentration were significantly enhanced. Significant increase in root growth was also noticed through the

increase in surface area, root volume, and the number of root tips. With the introduction of the bacterium, the IAA concentration in the soil was significantly increased. Spaepen, Dobbelaere, Croonenborghs, & Vanderleyden, 2008 studied the effect of bacteria produced indole-3-acetic acid (IAA) on wheat plant. *Azospirillum brasilense* is a very important rhizobacteria which is known to produce auxin (Spaepen et al., 2007) and is likely the most studied of the plant growth promoting bacteria. Wheat plants were inoculated with the wild type strain *Azospirillum brasilense Sp245*, resulting in an increase in root hair formation, plant development, dry weight yield and changes in wheat root morphology (Spaepen et al., 2008). All these effects were credited to auxin. Carmen Bianco & Defez, 2009 reported that bacteria produced IAA can confer protection against stresses such as; salt, acidity and UV. IAA helps bacteria to thrive well in the plant environment by adapting to stress conditions (C. Bianco et al., 2006). Hence, IAA production by these bacteria serves as an advantage in their environment (Kim et al., 2011). Thus there is substantial evidence for positive effects of auxin on plant growth and development. .

Spatial Arrangement: The soil system can be divided up into three spatially separate areas, namely: the bulk soil, the rhizosphere, and the endorhizosphere. The bulk soil is where bacteria can acclimate by the formation of resting or dormant cells such as spores, dwarf cells or cysts (Roszak & Colwell, 1987). The bulk soil has been shown to contain as many as 1 million different species per gram of soil (Gans, Wolinsky, & Dunbar, 2005). The rhizosphere is the narrow area surrounded and influenced by the plant root. The rhizosphere is considered to be one of the most biologically active ecosystems in the world (Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moënne-Loccoz, 2009). The rhizosphere is made up of diverse organisms namely bacteria, protozoa, algae, viruses, fungi,

oomycetes, nematodes, archaea, and antropods (Raaijmakers et al., 2009). Organisms in the rhizosphere utilizes the large amount of nutrient (exudates, border cells, mucilage) released by the plant (Mendes et al., 2013). On the other hand, plant stimulate beneficial microorganisms that may express traits that are important to plant growth and development (Cook et al., 1995). Some rhizosphere organisms such as nitrogen-fixing bacteria, plant growth promoting rhizobacteria (PGPR), mycorrhizal fungi, biocontrol microorganisms, selected protozoa and mycoparasitic fungi have been demonstrated to be beneficial to plant growth and development (Mendes et al., 2013). The endorhizosphere is comprised of the interior part of the root where fungi, bacteria and other microorganisms are in direct contact with the plant processes and begin to create a beneficial relationship (Kloepper, Schippers, & Bakker, 1992). Of the three the endorhizosphere should show the most intimate connection to plant processes.

Large and Small Colonies and Bacteria Growth: Bacteria growth is dependent on the availability of substrates, growth signaling compounds and the appropriate environmental conditions. If auxin is a signaling molecule for bacteria, it may have some effect on bacteria growth rates. The growth of bacteria can be modeled in four phases, i.e lag phase, log phase, stationary phase and death phase (Novick, 1955) (Skarstad, Steen, & Boye, 1983). The lag phase is the first developmental process in which bacteria adapt themselves to environmental conditions. During this period the cells may be synthesizing enzymes, proteins, RNA and increasing in metabolic activity. Several factors are responsible for the length of the lag phase period including the time required to repair physical damage or respond to shock to changes in new environment, the amount of the inoculum, the time required for synthesis of new enzymes that are necessary to metabolize new substrates

present in the medium (Novick, 1955). The log phase of growth is a period in which cells are dividing regularly by binary fission and growing by geometric progression. The cells constantly divide whose growth rate is based on the composition of the growth medium and the conditions of incubation (Novick, 1955). Stationary phase occurs when the level where the available nutrient is insufficient to sustain net growth rates. It is characterized by an accumulation of inhibitory metabolites or metabolic end product. The stationary phase results where there is a limitation of essential nutrient, and/or the formation of an inhibitory product such as a selected organic acid. Bacteria that produce secondary metabolites, such as antibiotics, often do so during the stationary phase of the growth cycle. It is during the stationary phase that spore-forming bacteria convert to the sporulation process (Novick, 1955). During the stationary phase life and death rates are in balance. The Death phase after the stationary phase when viable cell population declines. During the death phase, the number of viable cells decreases geometrically, essentially the reverse of growth during the log phase (Novick, 1955).

Bacteria growth rates during exponential phase, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more for slow growing bacteria (Zwietering, Jongenburger, Rombouts, & van 't Riet, 1990) (Skarstad et al., 1983). For most known bacteria that can be cultured, generation times range from about 15 minutes to 1 hour (Zwietering et al., 1990). Symbionts such as *Rhizobium* tend to have longer generation times. Many lithotrophs, such as the nitrifying bacteria, also have long generation times. Some bacteria that are pathogens, such as *Mycobacterium*

tuberculosis and *Treponema pallidum*, have especially long generation times, and this is thought to be an advantage in their virulence.

REFERENCES

- Al-idani, M. S. H. (2011). *Characterization of Auxin Producing Rhizobacteria in Wheat Plant Differing In Shoot Biomass*. (Master of Science), Oklahoma State University.
- Allan, M. (1977). *Darwin and his flowers: the key to natural selection* (Vol. 1): London: Faber 318p.-Illus., col. illus., maps, ports.. Icones, Maps. Geog.
- Amellal, N., Burtin, G., Bartoli, F., & Heulin, T. (1998). Colonization of Wheat Roots by an Exopolysaccharide-Producing *Pantoea agglomerans* Strain and Its Effect on Rhizosphere Soil Aggregation. *Applied and Environmental Microbiology*, 64(10), 3740-3747.
- Arroo, R., Develi, A., Meijers, H., Van de Westerloo, E., Kemp, A., Croes, A., & Wullems, G. (1995). Effects of exogenous auxin on root morphology and secondary metabolism in *Tagetes patula* hairy root cultures. *Physiologia Plantarum*, 93(2), 233-240.
- Balkovič, J., van der Velde, M., Skalský, R., Xiong, W., Folberth, C., Khabarov, N., . . . Obersteiner, M. (2014). Global wheat production potentials and management flexibility under the representative concentration pathways. *Global and Planetary Change*, 122(0), 107-121.
- Beeckman, T., Burssens, S., & Inzé, D. (2001). The peri-cell-cycle in Arabidopsis. *Journal of Experimental Botany*, 52(suppl 1), 403-411.
- Bianco, C., & Defez, R. (2009). *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. *Journal of Experimental Botany*, 60(11), 3097-3107.
- Bianco, C., Imperlini, E., Calogero, R., Senatore, B., Amoresano, A., Carpentieri, A., . . . Defez, R. (2006). Indole-3-acetic acid improves *Escherichia coli*'s defences to stress. *Archives of Microbiology*, 185(5), 373-382.
- Bisseling, T., Dangl, J. L., & Schulze-Lefert, P. (2009). Next-Generation Communication. *Science*, 324(5928), 691.
- Brandl, M. T., & Lindow, S. E. (1997). Environmental Signals Modulate the Expression of an Indole-3-Acetic Acid Biosynthetic Gene in *Erwinia herbicola*. *Molecular Plant-Microbe Interactions*, 10(4), 499-505.
- Casimiro, I., Marchant, A., Bhalerao, R. P., Beeckman, T., Dhooge, S., Swarup, R., . . . Bennett, M. (2001). Auxin Transport Promotes Arabidopsis Lateral Root Initiation. *The Plant Cell*, 13(4), 843-852.
- Chou, H.-M., Bundock, N., Rolfe, S. A., & Scholes, J. D. (2000). Infection of *Arabidopsis thaliana* leaves with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. *Molecular plant pathology*, 1(2), 99-113.
- Cook, R. J., Thomashow, L. S., Weller, D. M., Fujimoto, D., Mazzola, M., Bangera, G., & Kim, D. S. (1995). Molecular mechanisms of defense by rhizobacteria against root disease. *Proceedings of the National Academy of Sciences*, 92(10), 4197-4201.
- Costacurta, A., Mazzafera, P., & Rosato, Y. B. (1998). *Indole-3-acetic acid biosynthesis by Xanthomonas axonopodis pv. citri is increased in the presence of plant leaf extracts* (Vol. 159).

- Dakora, F., & Phillips, D. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, 245(1), 35-47.
- Darwin, C. (1888). The 'Power of movement in plants.'--1880. In F. Darwin (Ed.), *The life and letters of Charles Darwin, including an autobiographical chapter, Vol 3 (7th thousand rev.)* (pp. 329-338). London, England: John Murray.
- Depuydt, S., Trenkamp, S., Fernie, A. R., Elftieh, S., Renou, J.-P., Vuylsteke, M., . . . Vereecke, D. (2009). An Integrated Genomics Approach to Define Niche Establishment by *Rhodococcus fascians*. *Plant Physiology*, 149(3), 1366-1386. doi: 10.1104/pp.108.131805
- Dodd, I. C., Zinovkina, N. Y., Safronova, V. I., & Belimov, A. A. (2010). Rhizobacterial mediation of plant hormone status. *Annals of Applied Biology*, 157(3), 361-379.
- Doehlemann, G., Wahl, R., Vranes, M., de Vries, R. P., Kämper, J., & Kahmann, R. (2008). Establishment of compatibility in the *Ustilago maydis*/maize pathosystem. *Journal of Plant Physiology*, 165(1), 29-40.
- Domagalska, M. A., & Leyser, O. (2011). Signal integration in the control of shoot branching. *Nat Rev Mol Cell Biol*, 12(4), 211-221.
- Dubrovsky, J. G., Sauer, M., Napsucially-Mendivil, S., Ivanchenko, M. G., Friml, J., Shishkova, S., . . . Benková, E. (2008). Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proceedings of the National Academy of Sciences*, 105(25), 8790-8794.
- Egamberdieva, D. (2008). Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil. *Turkish Journal of Biology*, 32(1), 9-15.
- Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil. *Science*, 309(5739), 1387-1390.
- Haub, C., Gribble, J., & Jacobsen, L. (2011). World Population Data Sheet 2011. *Population Reference Bureau, Washington*.
- Herbers, K., Takahata, Y., Melzer, M., Mock, H. P., Hajirezaei, M., & Sonnewald, U. (2000). Regulation of carbohydrate partitioning during the interaction of potato virus Y with tobacco. *Molecular plant pathology*, 1(1), 51-59.
- Hussain, A., & Hasnain, S. (2011). Interactions of bacterial cytokinins and IAA in the rhizosphere may alter phytostimulatory efficiency of rhizobacteria. *World Journal of Microbiology and Biotechnology*, 27(11), 2645-2654.
- Jiang, Y., Xu, J., Zhao, K., Wei, H., Sun, J., Zhang, H., . . . Guo, B. (2015). Root system morphological and physiological characteristics of indica-japonica hybrid rice of Yongyou series. *Acta Agronomica Sinica*, 41(1), 89-99.
- Karas, I., & McCully, M. (1973). Further studies of the histology of lateral root development in *Zea mays*. *Protoplasma*, 77(2-3), 243-269.
- Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, 96(3), 473-480.
- Kim, Y. C., Leveau, J., McSpadden Gardener, B. B., Pierson, E. A., Pierson, L. S., & Ryu, C.-M. (2011). The Multifactorial Basis for Plant Health Promotion by Plant-Associated Bacteria. *Applied and Environmental Microbiology*, 77(5), 1548-1555. doi: 10.1128/aem.01867-10

- Kloepper, J. W., Schippers, B., & Bakker, P. (1992). Proposed elimination of the term endorhizosphere. *Phytopathology*, 82(7), 726-727.
- Kobayashi, M., Suzuki, T., Fujita, T., Masuda, M., & Shimizu, S. (1995). Occurrence of enzymes involved in biosynthesis of indole-3-acetic acid from indole-3-acetonitrile in plant-associated bacteria, *Agrobacterium* and *Rhizobium*. *Proceedings of the National Academy of Sciences*, 92(3), 714-718.
- Kremer, R. J., Begonia, M. F. T., Stanley, L., & Lanham, E. T. (1990). Characterization of Rhizobacteria Associated with Weed Seedlings. *Applied and Environmental Microbiology*, 56(6), 1649-1655.
- Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., . . . Laplaze, L. (2013). Lateral root development in Arabidopsis: fifty shades of auxin. *Trends in Plant Science*, 18(8), 450-458.
- Lee, J.-H., & Lee, J. (2010). *Indole as an intercellular signal in microbial communities* (Vol. 34).
- Liu, P., & Nester, E. W. (2006). Indoleacetic acid, a product of transferred DNA, inhibits vir gene expression and growth of *Agrobacterium tumefaciens* C58. *Proceedings of the National Academy of Sciences of the United States of America*, 103(12), 4658-4662.
- MacIsaac, S. A., Sawhney, V. K., & Pohorecky, Y. (1989). Regulation of lateral root formation in lettuce (*Lactuca sativa*) seedling roots: Interacting effects of α -naphthaleneacetic acid and kinetin. *Physiologia Plantarum*, 77(3), 287-293.
- Maitra, N., & Sen, S. P. (1987). Hormonal Regulation of Source-sink Relationship: Effect of Hormones on Excised Source and Sink Organs of Cereals. *Plant and Cell Physiology*, 28(6), 1005-1012.
- Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *Fems Microbiology Reviews*, 37(5), 634-663.
- Mole, B. M., Baltrus, D. A., Dangl, J. L., & Grant, S. R. (2007). Global virulence regulation networks in phytopathogenic bacteria. *Trends in Microbiology*, 15(8), 363-371.
- Morita, M. T., & Tasaka, M. (2004). Gravity sensing and signaling. *Current Opinion in Plant Biology*, 7(6), 712-718.
- Nakbanpote, W., Panitlurtumpai, N., Sangdee, A., Sakulpone, N., Sirisom, P., & Pimthong, A. (2013). Salt-tolerant and plant growth-promoting bacteria isolated from Zn/Cd contaminated soil: identification and effect on rice under saline conditions. *Journal of Plant Interactions*, 9(1), 379-387.
- Novick, A. (1955). Growth of bacteria. *Annual Reviews in Microbiology*, 9(1), 97-110.
- Patten, C. L., & Glick, B. R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*, 42(3), 207-220.
- Patten, C. L., & Glick, B. R. (2002). Role of *Pseudomonas putida* Indoleacetic Acid in Development of the Host Plant Root System. *Applied and Environmental Microbiology*, 68(8), 3795-3801.
- Peer, W. A., Blakeslee, J. J., Yang, H. B., & Murphy, A. S. (2011). Seven Things We Think We Know about Auxin Transport. *Molecular Plant*, 4(3), 487-504.

- Perley, J. E., & Stowe, B. B. (1966). ON ABILITY OF TAPHRINA DEFORMANS TO PRODUCE INDOLEACETIC ACID FROM TRYPTOPHAN BY WAY OF TRYPTAMINE. *Plant Physiology*, 41(2), 234-&.
- Raaijmakers, J., Paulitz, T., Steinberg, C., Alabouvette, C., & Moëgne-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, 321(1-2), 341-361.
- Ravari, S. B., & Heidarzadeh, N. (2014). Isolation and characterization of rhizosphere auxin producing Bacilli and evaluation of their potency on wheat growth improvement. *Archives of Agronomy and Soil Science*, 60(7), 895-905.
- Roszak, D. B., & Colwell, R. R. (1987). Survival strategies of bacteria in the natural environment. *Microbiological Reviews*, 51(3), 365-379.
- Sarwar, M., & Kremer, R. (1995). Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. *Plant and Soil*, 172(2), 261-269.
- Sato, E. M., Hijazi, H., Bennett, M. J., Vissenberg, K., & Swarup, R. (2014). New insights into root gravitropic signalling. *Journal of Experimental Botany*.
- Scharte, J., SchÖN, H., & Weis, E. (2005). Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. *Plant, Cell & Environment*, 28(11), 1421-1435.
- Schroth, L. (1986). Influence of bacteria sources of indol-3-acetic acid on root elongation of sugar beet. *Phytopathology*, 76, 386-389.
- Skarstad, K., Steen, H. B., & Boye, E. (1983). Cell cycle parameters of slowly growing *Escherichia coli* B/r studied by flow cytometry. *Journal of Bacteriology*, 154(2), 656-662.
- Spaepen, S., Dobbelaere, S., Croonenborghs, A., & Vanderleyden, J. (2008). Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. *Plant and Soil*, 312(1-2), 15-23.
- Spaepen, S., & Vanderleyden, J. (2011). Auxin and Plant-Microbe Interactions. *Cold Spring Harbor Perspectives in Biology*, 3(4).
- Spaepen, S., Vanderleyden, J., & Remans, R. (2007). *Indole-3-acetic acid in microbial and microorganism-plant signaling* (Vol. 31).
- Stes, E., Vandeputte, O. M., El Jaziri, M., Holsters, M., & Vereecke, D. (2011). A Successful Bacterial Coup d'État: How *Rhodococcus fascians* Redirects Plant Development. *Annual Review of Phytopathology*, 49(1), 69-86.
- Swarup, R., Kramer, E. M., Perry, P., Knox, K., Leyser, H. M. O., Haseloff, J., . . . Bennett, M. J. (2005). Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat Cell Biol*, 7(11), 1057-1065.
- Taylor, R. D., & Koo, W. W. (2015). 2015 Outlook of the U.S. and world wheat industries, 2015-2024. *Agribusiness & Applied Economics Report - Department of Agribusiness and Applied Economics, North Dakota State University*(738), v + 23 pp.
- Teale, W. D., Paponov, I. A., & Palme, K. (2006). Auxin in action: signalling, transport and the control of plant growth and development. *Nat Rev Mol Cell Biol*, 7(11), 847-859.

- Theunis, M., Kobayashi, H., Broughton, W. J., & Prinsen, E. (2004). Flavonoids, NodD1, NodD2, and Nod-Box NB15 Modulate Expression of the y4wEFG Locus That Is Required for Indole-3-Acetic Acid Synthesis in *Rhizobium* sp. strain NGR234. *Molecular Plant-Microbe Interactions*, *17*(10), 1153-1161.
- Valls, M., Genin, S., & Boucher, C. (2006). Integrated Regulation of the Type III Secretion System and Other Virulence Determinants in *Yersinia enterocolitica*. *PLoS Pathog*, *2*(8), e82.
- Waters, C. M., & Bassler, B. L. (2005). QUORUM SENSING: Cell-to-Cell Communication in Bacteria. *Annual Review of Cell and Developmental Biology*, *21*(1), 319-346.
- Went, F. W., & Thimann, K. V. (1937). *Phytohormones*: MacMillan Company, New York.
- Wright, D. P., Baldwin, B. C., Shephard, M. C., & Scholes, J. D. (1995). Source-sink relationships in wheat leaves infected with powdery mildew. I. Alterations in carbohydrate metabolism. *Physiological and Molecular Plant Pathology*, *47*(4), 237-253.
- Xu, G.-W., & Gross, D. C. (1988). Evaluation of the Role of Syringomycin in Plant Pathogenesis by Using Tn5 Mutants of *Pseudomonas syringae* pv. *syringae* Defective in Syringomycin Production. *Applied and Environmental Microbiology*, *54*(6), 1345-1353.
- Xu, L., Xu, W. S., Jiang, Y., Hu, F., & Li, H. X. (2015). Effects of Interactions of Auxin-Producing Bacteria and Bacterial-Feeding Nematodes on Regulation of Peanut Growths. *Plos One*, *10*(4).
- Yang, S., Zhang, Q., Guo, J., Charkowski, A. O., Glick, B. R., Ibekwe, A. M., . . . Yang, C.-H. (2007). Global Effect of Indole-3-Acetic Acid Biosynthesis on Multiple Virulence Factors of *Erwinia chrysanthemi* 3937. *Applied and Environmental Microbiology*, *73*(4), 1079-1088.
- Yue, J., Hu, X., & Huang, J. (2014). Origin of plant auxin biosynthesis. *Trends in Plant Science*, *19*(12), 764-770.
- Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & van 't Riet, K. (1990). Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology*, *56*(6), 1875-1881.

CHAPTER II

THE RELATIONSHIP BETWEEN AUXIN PRODUCTION CAPACITY BY RHIZOBACTERIA AND WHEAT BIOMASS

INTRODUCTION

Auxin (indole-3-acetic acid, IAA) is one of the most important plant hormones known, functioning in the regulation of plant growth and development. Interestingly, bacteria and other microorganisms also produce auxin. In fact in a survey, 80% of all rhizosphere bacteria were auxin producers (Patten & Glick, 1996). The functional relevance of bacterial auxin production to plant growth and development and bacteria growth and survival is unknown.

Auxin affects almost every aspect of plant growth and development including vascular bundle formation, vascular tissue differentiation, apical dominance, initiation of adventitious and lateral roots, elongation and growth in stems and root, cell division, and tropic responses to gravity and light (Peer, Blakeslee, Yang, & Murphy, 2011; Sánchez-Rodríguez, Rubio-Somoza, Sibout, & Persson, 2010). The fact that microorganisms whose evolutionary development predate that of plants produce auxin suggests that plants retained the auxin signaling system in their evolutionary development from single celled algae to multicellular complex flowering plants. This may suggest that originally bacteria produced auxin served as an essential integrative signaling compound within the bacteria

community. An understanding of auxins functional relevance will lead to a better understanding on how the plant-bacteria community grows and develops.

It is widely assumed that bacteria produced auxin impacts plant growth and development. Most of the evidence involves application of auxin producing bacteria, use of auxin deficient mutants, and application of endogenous auxin to growing plants (Costacurta & Vanderleyden, 1995) (Patten & Glick, 1996), (A. Khalid, Arshad, & Zahir, 2004). M. Khalid, Zahir, Waseem, & Arshad, 1999 and Ali, Sabri, Ljung, & Hasnain, 2009 reported that bacteria produced auxin can be used to increase crop yield, by enhancing root proliferation through improved mineral uptake. Díaz-Zorita & Fernández-Canigia, 2009 noted that a bacteria strain *Azospirillum. brasilense* Az39 used to inoculate wheat roots, caused a shortening of the wheat plant's primary root and an increase in lateral root and root hair, which resulted to an apparent increase in root surface area and nutrient uptake. These increases were accompanied by greater shoot biomass, increased growth and yield under agronomic conditions (Díaz-Zorita & Fernández-Canigia, 2009). The rhizobacteria *Azospirillum brasilense* and *Enterobacter cloacae* are known promoters of root development presumably mediated through the production of auxin (Patten & Glick, 2002). In separate studies the auxin producing bacteria *Azospirillum. brasilense* CBG497 was used to inoculate maize in the Easpur loam and *A. lipoferum* 4B was used to inoculate rice under field condition, both showing a significant increase in overall yield (Bally et al., 1983).

On the other hand, Mohammad Al-idani 2011 showed a negative relationship between auxin production capacity and plant biomass: bacteria from high biomass plants

showed low auxin production while bacteria from low biomass plants show high auxin production. Morris, 1995 also reported the negative effect of auxin producing bacteria, such as *Agrobacterium tumefaciens*, *Pseudomonas savastanoi*, *Erwinia herbicola*, and *Rhodococcus* on plant growth. *Agrobacterium* over-produced auxin inducing plant tumors called galls leading to abnormal shoot morphology (Mole, Baltrus, Dangl, & Grant, 2007). Many pathogens including *P. syringae*, can also produce auxin (Eric Glickmann et al., 1998) (Spaepen & Vanderleyden, 2011). Fett, Osman, & Dunn, 1987 showed that plant pathogenic *Pseudomonas* and *Xanthomonads* are capable of producing auxin *in vivo* when induced by L- tryptophan. Bacteria auxin is involved in causing various infectious plant diseases such as soft rot, leaf wilt, several blight diseases caused by *Erwinia chrysanthemi* (Yang et al., 2007), and gall diseases by *Pantoea agglomerans* (Chalupowicz, Barash, Panijel, Sessa, & Manulis-Sasson, 2009). Thus there is plenty of evidence showing negative side effects of bacteria produced auxin.

The term rhizosphere was first defined in 1904 by Lorenz Hiltner as “the soil compartment influenced by the root” (Hiltner, 1904). In essence it is also the soil that surrounds the root surface. Bacteria found in the rhizosphere are called rhizobacteria (van Loon, 2007). The rhizosphere is described as the most microbially active habitat in the soil system comprising the lysates from dead plant and microbial cells, plant mucilage and other plant exudates (Hartmann, Lemanceau, & Prosser, 2008). Plants releases many compounds into the rhizosphere, including carbohydrates, organic acids, amino acids, ectoenzymes and polysaccharides serving as carbon and energy substrates (Kloeppe et al., 1999) (Travis, Harsh Pal, Grotewold, & Vivanco, 2003). In the rhizosphere, organismal numbers generally increase as you go from the bulk soil to the root surface (rhizoplane),

through the rhizosphere. The rhizoplane is located on the root surface including the root epidermis and mucilage.

Many other rhizobacteria are known to colonize the inside of plant tissue termed the endorhizosphere, consisting of portions of the endodermis, cortex and the root hairs in which microorganism reside. Organisms residing in the endorhizosphere may have a mutually beneficial symbiotic relationship with the host stemming from increased nutrient use efficiency, provision of growth hormones, or plant protection from disease. In exchange the plant provides energy carbohydrates for microbial growth processes (Spaepen, Vanderleyden, & Remans, 2007).

Rationale of This Study: We hypothesize based on Al-idani 2011 that there is a negative correlation between auxin production capacity of rhizosphere and endorhizosphere colonizing bacteria and plant productivity. In this study we plan to both determine the direction and magnitude of this associations. We will isolate a large number of individual bacteria from the rhizosphere and endorhizosphere of wheat plants varying naturally in biomass, and then correlate the auxin production capacity to plant biomass. If we see that high auxin producers are found predominantly in high biomass plants and low auxin producers in low biomass plants, this will indicate a positive association, and the opposite relationship will indicated a negative relationship. Performing this analysis in the rhizosphere and the endorhizosphere will provide information for the effect of intimacy with the plant root in this relationship. The direction of the relationship may be either positive or negative as suggested in the introduction. The results presented here will provide insight into the functional association of bacteria produced auxin in the rhizosphere

and endorhizosphere to plant productivity. A large and significant correlation would suggest that auxin production capacity can be used as markers of productive plant-biomass associations.

Objectives:

1. Determine the relationship between auxin production capacity by rhizobacteria and plant biomass.
2. Determine if spatial proximity to the root affects the relationship between auxin production capacity and wheat biomass productivity.
3. Determine if soil types have an effect on auxin production capacity.
4. Determine the relationship between bacteria colony size and auxin production capacity of rhizosphere bacteria *in vitro*

MATERIAL AND METHODS

Plant Management: Wheat Plants var Duster were grown in two soil types: a Teller fine sandy loam, pH 6.7, from the OSU Perkins field station and the other an Easpur loam, pH 7.8, from the Stillwater Field Station. Both soils have a previous history of wheat production. Prior to planting, the soils were homogenized in a mixer for at least 10 min and then fertilized by adding ammonium nitrate to an equivalent of 78 kg of N/ha. The mixed soil were then be evenly distributed among 96, 1.6 liter Mini-Treepots (Stuewe and Sons, TP49, Tangent OR). Duster variety was planted and watered at 2.5 cm depth in both soils, 3 seeds per pot. After emergence, seedlings were thinned so that only one seedling remained by selecting the most centrally located seedling to avoid selection bias. Plants were watered evenly when the soil moisture approached the dry range as determined by the soil moisture meter (Etekcity Inc, USA). Mini-Treepots were stored in trays 16 pots per tray, and tray location were re-randomized seven times throughout the growth season to sample the variation within the Easpur loam environment. Plants were grown during January through March at an average temperature set at 22⁰ C and a supplemented photoperiod of 14 hrs. Plants were harvested at the Feekes stage 9 prior to boot formation.

Harvest: The wheat plants grown in both Teller and Easpur soils were harvested separately one week apart. Plants were gently removed from pots, and the non-rhizosphere soil were eliminated by attaching shoot-root system a chord and dropping the plant 60 cm three times. The shoot and the root with clinging rhizosphere soil were cut and weighted separately. The root with rhizosphere soil was added to a 250 ml canning jar with 100 ml of autoclaved 0.1% sodium pyrophosphate pH 6.5. The jars were sealed with a lid and agitated at 250 rpm for five minute. After shaking, 1 ml of rhizosphere soil solution were

transferred to 1.5 ml micro-centrifuge tube containing 500 µl of autoclaved 30% glycerol + 0.1 x TSB. The tubes were mixed and immediately placed on ice. This constituted the rhizosphere fraction. The root surfaces were rinsed three times in deionized water and then cleaned by shaking at 250 rpm for 5 minutes in a solution of 100 ml of 0.2% Palmolive dish soap without antibacterial ingredients (Colgate Palmolive Inc, USA). The jar with root was rinsed with deionized water and sanitized with 70% ethanol. After sanitization, 200 ml of deionized water was added with 1 ml of 0.1% sodium pyrophosphate pH 6.5 in a Waring blender and homogenizing at high speed for 1 minute to release bacteria from inside the root tissues. This constituted the endorhizosphere fraction. The blended solutions were sampled while stirring and placed in a 1.5 ml microcentrifuge tube containing 500 µl of autoclave 30% glycerol + 0.1x TSB, mixed and place on ice. All samples were placed in a -21⁰ C freezer for later analysis. A total of 96 samples from rhizosphere and endorhizosphere were collected and analyzed.

Culturable Library Development for Rhizosphere and Endorhizosphere: Bacteria from the rhizosphere and endorhizosphere from wheat plants grown in the Teller soil and bacteria from the rhizosphere of wheat plants grown in Easpur soil were purified and collected as individual isolates. A total of 96 µl of rhizosphere extract was diluted in ten-fold increments from 10⁻¹ to 10⁻⁶ dilutions in PBS + 5.2 mg nystatin in a deep well plate by serial mixing using a pipettor. All procedures were performed under a laminar flow hood under sterile conditions. For endorhizosphere extracts dilutions of 10⁻¹ to 10⁻⁴ were performed due to anticipated lower numbers of bacteria compared to the rhizosphere. The dilutions were uniformly spread plated on top of 0.1X tryptic soy agar (TSA) using a sterile spreader bar with rotation. The plates were incubated for 4 days. Individual colonies were

randomly picked using a sterile loop and quadruple streaked across another 0.1X TSA plate to separate bacteria colonies so that they can be individually selected with minimal contamination. After 4 days of culture growth both large (> 2mm) and small (< 2mm) colonies were collected and labeled. Selected isolates were transferred to 4 ml of 1X TSB in a 13 mm glass tube with aeration cap using a sterile loop. The isolates were allowed to grow for 4 days with continuous shaking at 250 rpm. A 50 µl sample of culture was combined with 50 µl of freezer media in a 96 well archive plate sealed with plastic aeration film (Bemis Co. Inc. Neenah, WI, USA). The archive plates were stored at -20⁰ C.

Auxin Growth Promotion: After the initial growth 10 µl of culture was transferred to 1 ml of 1X TSB contained in a 96 deep well plate. The plate was sealed with sterile aeration tape and the plate were shaken at 250 rpm for 3 days. The plate were centrifuged to pellet the cells at 3200 rpm for 10 min in an IEC centrifuge (IEC size 2, Model K centrifuge, Star Industry, CA, USA). A total of 150 µl of supernatant was carefully removed to an auxin assay 96 well plate avoiding the pellet. Also, 150 µl of a set of auxin standards ranging from 0 to 50 µg /ml was added to each plate. The blank absorbance of the plate was read using a Sunrise Tecan plate reader, (Tecan Inc, Switzerland) at 540 nm prior to adding the sample. A total of 100 µl of Solution 1 (Glickmann, 1995) was added to the assay plate and the solutions carefully mixed by pipetting. Solution 1 was prepared by adding 84 mls of concentrated H₂SO₄, 116 mls of deionized water, and 2.4g of FeCl₃. The plate was shaken on an orbital shaker for 45 minutes at room temperature to incubate the reaction and permit the colorimetric transformation. After 45 minutes, absorbance at 540 nm was read for both sample and standards. After centrifugation of the deep well plate the

supernatant was poured out retaining the cellular pellet at the bottom of the wells. A total of 1 ml of deionized water was added to each well and the plates were shaken for 5 minutes at 250 rpm to re-suspend the pellets. The absorbance of the solution minus the blank plate was recorded at 595 nm from 200 μ l of re-suspended cells reflecting overall bacterial abundance at the time of assay.

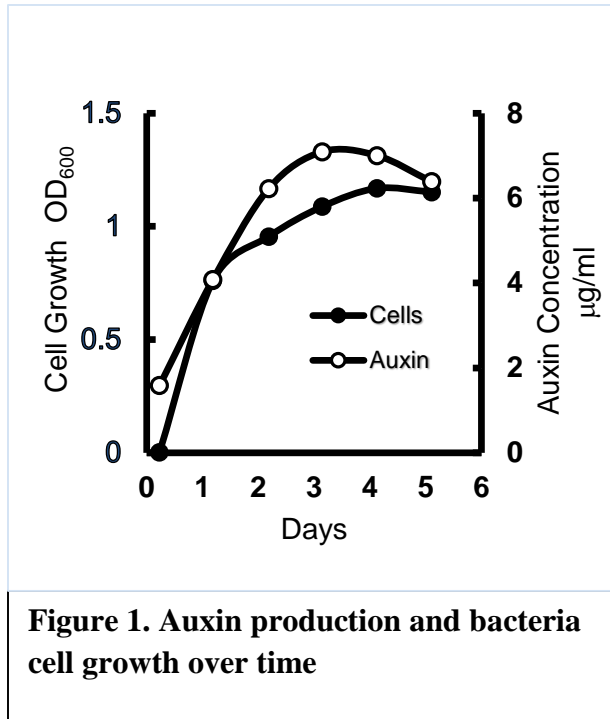
Auxin Production Capacity and Analysis: A total of 4320 individual bacteria from the rhizospheres, and endorhizospheres of 20 wheat plants of varying biomass, 72 isolates per plant were obtained. A total of thirty six isolates randomly selected were small colonies while the remaining thirty six were large colonies. The process ensured unbiased selection of isolates by placing the culture plate on top of a paper marked with circles. All isolates that fell within a given circle were chosen from a given culture plate. All isolates were assayed for auxin production capacity as detailed above. Every isolate was grown in four replicate cultures. The auxin production capacity was assayed three times as indicated above for each of the four replicate cultures, for a total of 12 data points per isolate, calculated in an Excel spreadsheet based on the auxin standard curve using least square regression. The overall average production capacity of isolates from the rhizosphere and endorhizosphere, were determined in the same spreadsheet. Differences in average auxin production capacity among rhizosphere and endorhizosphere communities were determined using full factorial analysis of variance (ANOVA, SAS-JMP Pro version 11.0) and Tukeys multiple comparisons with a significance level of $p \leq 0.05$. The assumptions of normality were determined using Shapiro-Wilk statistical test.

Auxin Production Capacity and Bacterial Growth: A total of 16 wheat plants were grown and harvested as above. From each plant, 36 isolates were randomly selected for a total of 576 isolates. These were tested for auxin production capacity and cell growth every day from 0 to day five as indicated above. Results were analyzed on an Excel spreadsheet and statistically analyzed using (SAS-JMP v. 11.0). The correlation between auxin production capacity and cell growth over the six day period was analyzed by least square regression.

RESULT AND DISCUSSION

Plant Growth: A total of 96 wheat plants were planted under greenhouse conditions in a Teller and Easpur soils. Twenty plants were randomly selected from both soils for isolating bacteria from the rhizosphere and endorhizosphere, and screened for large and small colony size, and auxin production capacity. A total of 4320 bacteria were isolated and characterized. Shoot biomass ranged from 2.83 g to 10.52 g in wheat grown in the Easpur soil with an average of 8.06 g, and 0.61 to 12.12 g with an average of 9.02 g in the Teller soil. Overall average shoot biomass was significantly different between the two soil types (p value 0.0001). The difference in average shoot weight may have been due to pH differences between the two soils with the Teller soil showing a lower pH and a higher biomass yield, while the reverse is true for the Easpur loam soil. Optimum pH for growing wheat is from neutral to slightly acidic (Mullins & Sikora, 1994) favoring the Teller soil.

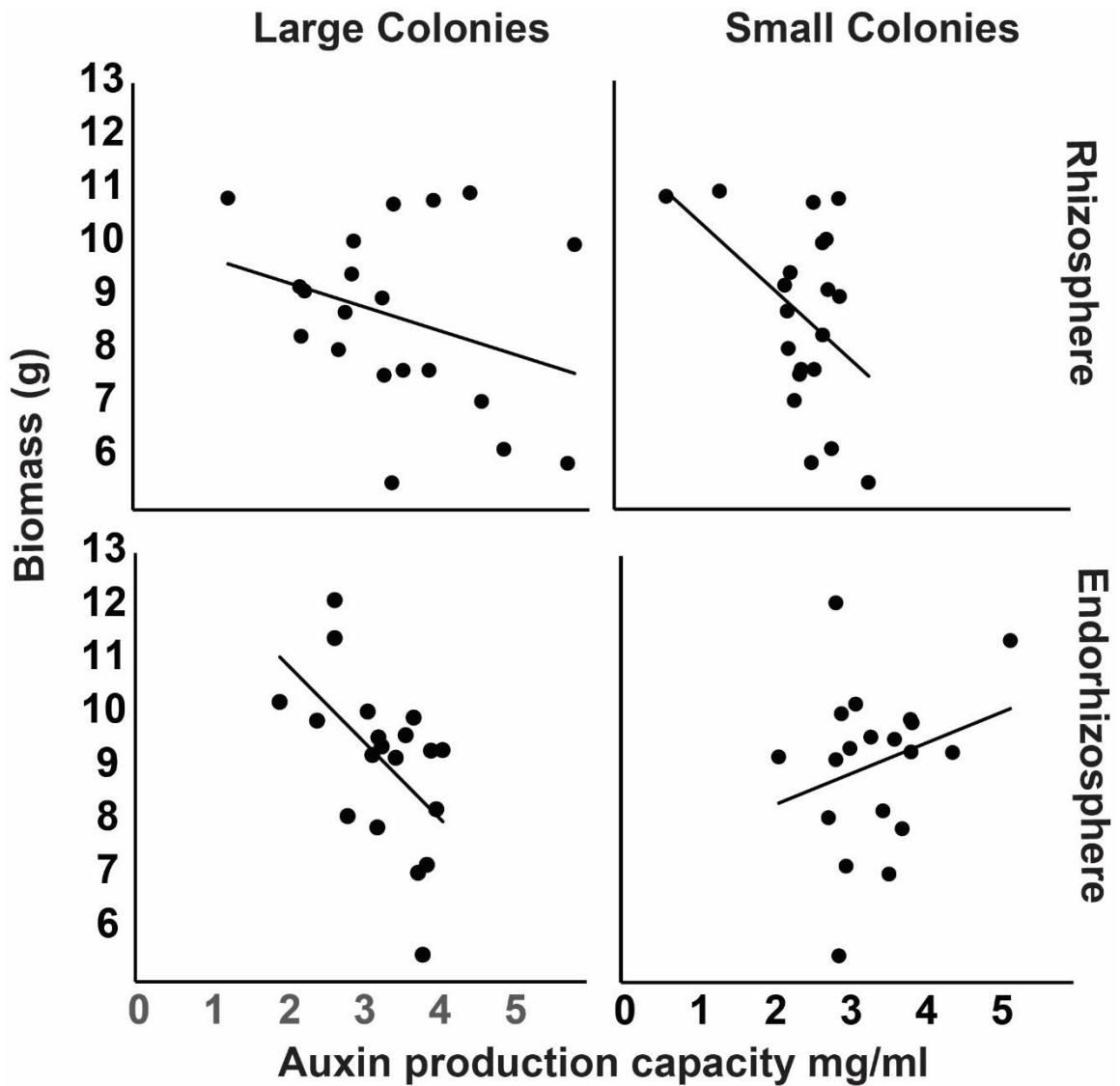
Auxin Production and Bacteria Cell Growth: Auxin is known as an essential element in plant cell growth, affecting both cell division and cell expansion. The role of auxin in bacterial communities is completely unknown. The relationship between auxin production and bacterial cell growth was examined over the same 5 day period using the auxin assay. *In vitro* auxin production was measured for 576 isolates to determine the optimal growth period prior to measuring auxin production capacity in all our isolates. A set of 16 plants were randomly selected and used to isolate bacteria, which were assayed for auxin production capacity over a 5 day period (Figure 1). Auxin levels increased to around 7 µg/ml from time zero to days 3 and 4. The maximum average auxin production by individual bacteria was found to be 7.09 µg/ml at day 3. Auxin production differed



statistically from days 0-2 and days 3-5. There was a small non-significant numerical decline after 3 days. Therefore, in all our later experiments we isolated bacteria grown *in vitro* after 3 to 4 days of growth.

Examining average cell growth in culture over time the result revealed that maximum cell growth was 1.17 absorbance units, which was recorded

at day 4. There was a significant difference in cell growth between 0-3 days compared to 4-5 days ($p \text{ value} \leq 0.0015$). The growth of bacteria corresponded very closely with the auxin production capacity over the 5 day period with an R^2 value of 0.98 based upon a one phase association model common between ligand and receptor interactions (Monine, Posner, Savage, Faeder, & Hlavacek, 2010). The strong correlation implies that auxin production by bacterial cells grown in culture is related to their community growth response.



	Large Colonies			Small Colonies		
	Slope	p value	R ²	Slope	p value	R ²
Rhizosphere	-0.45	0.178	0.099	-1.29	0.051	0.200
Endorhizosphere	-1.42	0.015	0.302	0.58	0.290	0.066

Figure 2. Correlation between auxin production capacity and plant biomass in Teller fine sandy loam soil.

Very little is positively known concerning the effect of bacteria produced auxin on plant growth and development (Spaepen & Vanderleyden, 2011). In the Teller soil, 20

plants were randomly selected with a maximum biomass of 10.94g and a minimum of 5.5 g and an average of 8.61g. From this experiment the large and small colony rhizosphere and endorhizosphere bacteria were extracted, cultured on 1/10x TSA, auxin production capacity determined and the correlations between biomass and auxin production capacity were revealed (Figure 2). Three out of the four slopes were generally negative with regard to biomass (y-axis) and auxin production capacity (x-axis) ranging from -1.42 for the large colony endorhizosphere bacteria to a slightly positive slope of 0.58 for endorhizosphere small colony bacteria. All of the slopes were negative for large colony bacteria. The only positive slope was from the small colony endorhizosphere bacteria. None of the slopes were significantly different from zero except for the large colony endorhizosphere bacteria (p value, 0.015) showing a negative value. The R^2 ranged from 0.30 to 0.07 among the four comparisons indicating little association between biomass and auxin production capacity. This data suggests that there is little and possibly a negative relationship between the auxin production capacity of bacteria associated with plant roots and biomass accumulation.

The endorhizosphere bacteria has a closer intimacy with the plant and so the relationship between auxin production capacity by these isolates and biomass was originally thought to be more positive. The results presented here showed very little if any difference between the endorhizosphere and rhizosphere as far as auxin production capacity influence on biomass. Al-idani 2011 showed a much stronger negative association between auxin production capacity and wheat biomass using a different soil system and analysis procedure compared with the results presented here. Al-idani 2011 categorized his plants into three biomass categories high, medium and low, and then correlated the biomass values to auxin production capacity. In this work isolates and plants from which they were

extracted were randomly selected and not categorized. The random selection may have resulted in a more powerful selected statistical analysis. This work is at least in partial agreement with Al-idani 2011 showing a negative association in most cases.

Auxin Production Capacity in Rhizosphere and Endorhizosphere: Isolation of individual bacteria for auxin production capacity was done from two spatially distinct regions in the Teller soil-plant interface namely; rhizosphere and endorhizosphere (Table 1).

Table 1. Auxin production capacity in the rhizosphere and endorhizosphere by large and small colony bacteria in the Teller fine sandy loam soil

	Large colony	Small colony	Average	R vs E*
<i>µg/ml</i>				
Rhizosphere	3.47 ± 0.18 ^a	2.48 ± 0.18 ^b	2.97 ± 0.13	p ≤ 0.046
Endorhizosphere	3.30 ± 0.18 ^a	3.39 ± 0.18 ^a	3.34 ± 0.13	
Average	3.39 ± 0.13	2.93 ± 0.13	3.15	
Large vs Small Colony	p ≤ 0.015			

Represents average of 720 isolates ± standard error

Letter (a,b) represent significant difference based on Tukeys HSD with a p value ≤ 0.05

*Rhizosphere vs Endorhizosphere Soil p value

The rhizosphere is the region located just within a few millimeters from the root surface (Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moënné-Loccoz, 2009), while the endorhizosphere is the region within the root itself (Kloepper, Schippers, & Bakker, 1992). Both contain extensive microbial communities (Gans, Wolinsky, & Dunbar, 2005). A total of 2880 bacteria were isolated from the rhizosphere soil and the endorhizosphere from a

set of 20 randomly selected plants. These were the same as the 4320 bacteria indicated above, but for only one soil system only. For each plant, 72 isolates for both endorhizosphere and rhizosphere were randomly selected and screened for auxin production capacity. Among 2880 within the two spatial regions (Rhizosphere and Endorhizosphere), isolates were also equally divided into two categories namely; large colony and small colony bacteria or 720 isolates for each combination. Overall average auxin production capacity was 3.15 $\mu\text{g/ml}$. Examining the average auxin production capacity across rhizosphere and endorhizosphere indicated that rhizosphere organisms showed a 11% lower auxin production capacity than endorhizosphere organisms. This difference was significant ($p \leq 0.046$). Examining the average auxin production capacity across large colony and small colony bacteria isolated from Teller soil, large colony bacteria exhibited a 14% greater auxin production capacity than slow growers. While these differences were statistically significant ($p \leq 0.015$) the actual numerical differences were slight overall but may be meaningful if one considers the aggregate effect from the whole community.

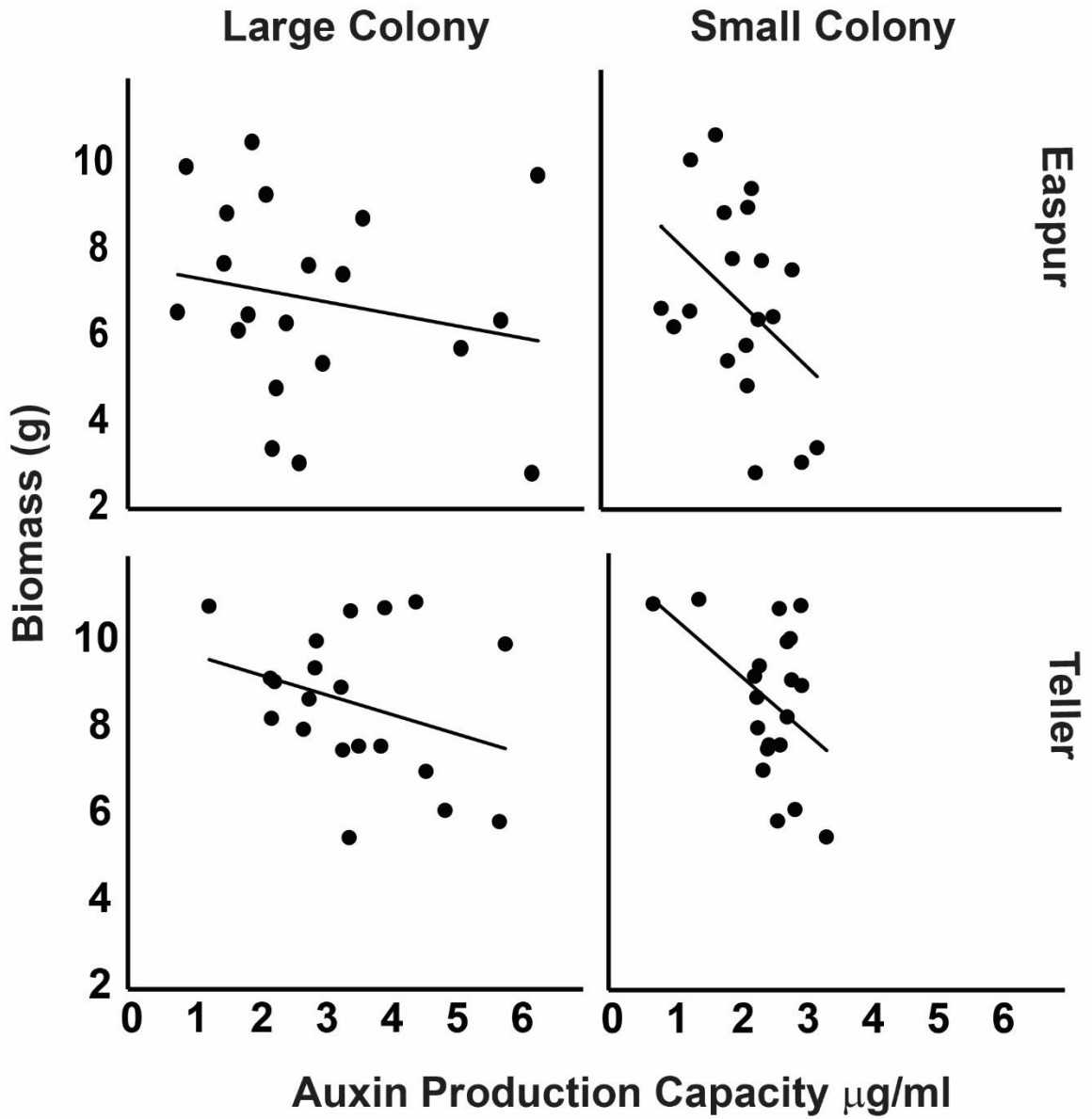
Examining the auxin production capacity of isolates among rhizosphere and endorhizosphere including large colony and small colony bacteria indicated that maximum auxin producing capacity was in the rhizosphere for large colony bacteria (3.47 $\mu\text{g/ml}$) while the minimum in the rhizosphere small colony bacteria (2.48 $\mu\text{g/ml}$). This difference was significant ($p \leq 0.05$). The endorhizosphere showed much less non-significant differences between large and small colony bacteria (3.30 vs 3.39 $\mu\text{g/ml}$). The overall maximum auxin production capacity from randomly recovered isolates was 14.05 $\mu\text{g/ml}$

from an endorhizosphere large colony while the lowest maximum was from the rhizosphere small colony bacteria at 5.31 $\mu\text{g/ml}$. The minimums for the most part were below the detection limit of the assay which was previously determined to be 0.4 $\mu\text{g/ml}$ (Al-idani, 2011).

Correlation Between Auxin Production Capacity and Plant Biomass Between Soil

Types: Two soils namely referred to as the Easpur loam and the Teller fine sandy loam soil were used to isolate large and small colony bacteria, which were tested for auxin production capacity. In the Easpur loam and Teller fine sandy loam soil, a set of 20 plants were randomly selected. The maximum biomass of the plants randomly selected from the Easpur loam soil was 10.52 g and a minimum of 2.83 g with an average of 6.86 g. From this experiment, the large and small bacteria from the rhizosphere of both Easpur loam and Teller fine sandy loam soils were extracted and the correlations between biomass and auxin production capacity were determined (Figure 3). Interestingly, all the four slopes were negative ranging from -1.44 from small colony bacteria to -0.28 from large colony bacteria from Easpur loam soil from the rhizosphere. None of the slopes were significantly different from zero though the small colony isolate from Teller fine sandy loam was close to being significant with a p value of 0.051. The R^2 ranged from 0.20 to 0.04 among the four comparisons indicating little association between biomass and auxin production capacity. This data supports the suggestion above that there is little and possibly a negative relationship between the auxin production capacity of bacteria associated with plant roots

and biomass accumulation among two contrasting soil types, which suggests that auxin production capacity may not be involved with growth promotions.



	Large Colony			Small Colony		
	Slope	p value	R ²	Slope	p value	R ²
Easpur	-0.28	0.383	0.042	-1.44	0.081	0.168
Teller	-0.45	0.178	0.099	-1.29	0.051	0.195

Figure 3. Correlation between Auxin Production Capacity and Plant Biomass in plants growing in Easpur loam and Teller fine sandy loam soil

Auxin Production Capacity in the Rhizosphere of Teller and Easpur Soils: Isolation of individual auxin producing rhizosphere bacteria was performed for two distinct soil types namely; Easpur loam and the Teller fine sandy loam Soil (Table 2).

Table 2. Auxin production capacity by large and small colony bacteria from plants growing in Easpur loam and Teller fine sandy loam soil.

	Large Colony	Small Colony	Average	G vs P*
	$\mu\text{g/ml}^*$			
Easpur loam Soil	$2.89 \pm 0.27^{\text{ab}}$	$2.33 \pm 0.27^{\text{b}}$	2.61 ± 0.19	$p \leq 0.183$
Teller fine sandy loam	$3.47 \pm 0.27^{\text{a}}$	$2.48 \pm 0.27^{\text{ab}}$	2.97 ± 0.19	
Average	3.18 ± 0.19	2.40 ± 0.19	2.79	
L vs S	$p \leq 0.005$			

Represents average of 720 isolates \pm standard error

Letter (a,b) represent significant difference based on Tukeys HSD with a p value ≤ 0.05

* Easpur loam vs Teller fine sandy loam Soil p value

The rhizosphere is a region associated with high microbial activities that is directly influenced by root secretion. A total of 1440 total bacteria were isolated from the rhizosphere of both soil from a set of 20 randomly selected plants. For each plants, 72 isolates were randomly selected and screened for auxin production capacity from the rhizosphere of both Teller fine sandy loam and Easpur loam soil. The 1440 bacteria from plants grown in the two soils were randomly selected from two groups namely; 720 large colony and 720 small colony bacteria. The overall average auxin production capacity for both the Teller and Easpur soils was 2.79 $\mu\text{g/ml}$. However, the average auxin production in the Teller soil was 14% higher than in the Easpur soil (2.98 vs 2.61) though the

difference was not significant ($p \leq 0.183$). Also, average auxin production significantly differed ($p \leq 0.005$) among large (3.18 $\mu\text{g/ml}$) and small (2.41 $\mu\text{g/ml}$) colony bacteria. Large colony bacteria from the Teller soil had a non-significant higher average auxin production capacity (3.47 $\mu\text{g/ml}$) than the Easpur soil (2.89 $\mu\text{g/ml}$). From the small colony bacteria, the Teller soil again had a non-significant higher average auxin production capacity at 2.48 $\mu\text{g/ml}$ than the small colony bacteria found in the Easpur loam soil which was 2.33 $\mu\text{g/ml}$. The maximum auxin production capacity which was found among large colony bacteria in the rhizosphere Easpur soil at 14.15 $\mu\text{g/ml}$.

The negative action of bacteria auxin on plant growth has yet to be fully appreciated. Our results suggest that bacteria auxin by itself is not a good indicator of growth promotion. In fact, bacteria produced auxin may be more associated with growth reduction as indicated by the results presented here in two separate experiments and by Alidani 2011. Many researchers use auxin as an indicator of growth promotion (A. Khalid et al., 2004) (Marques, Pires, Moreira, Rangel, & Castro, 2010) (Cassán et al., 2009). However the rationale for doing so is not firmly established. Recent studies proposed that in certain cases, growth promotion is more a function of auxin catabolism, than auxin production (Zúñiga et al., 2013) (Leveau & Lindow, 2005) suggesting the growth promotion is not completely a function of auxin synthesis and that a reduction in auxin is associated with plant growth. Also, Nakbanpote et al., 2013, suggested the negative effect of isolates on the growth of rice seedlings. Wheat plants were inoculated with known auxin producers causing a significant decrease in the germination rate for the wheat seedlings. Thus, bacteria produced auxin had a negative impact on germination. Other negative effects of auxin on plant health have been well documented. Application of exogenous

auxin is known to decrease resistance to disease (Navarro et al., 2006) (Zúñiga et al., 2013), many pathogens are known auxin producers (Eric Glickmann et al., 1998) (Remans, Spaepen, & Vanderleyden, 2006) and infection by pathogens is often followed by an increase in plant auxin levels (O'Donnell et al., 2003). Pathogens are even capable of co-opting auxin biosynthetic pathways in order to promote virulence and infection (Robert-Seilaniantz, Grant, & Jones, 2011). These reports call into question the proposition that auxin production by bacteria or other microorganisms is strictly equated with growth promotion.

Most research to date has focused on a few selected strains of auxin producing bacteria that are known to promote plant growth, or are pathogenic. Almost all of these bacteria are capable of producing auxin. In fact, one study found that 20-100% of all culturally isolated bacteria produced detectable levels of auxin, depending on the taxonomic unit studied (Ahmad, Ahmad, & Khan, 2008)(Patten & Glick, 1996). A. Khalid et al., 2004 noted that over 80% of the bacteria isolated from the rhizosphere are capable of producing auxin. Ali-dani 2011 found that 85% of all isolates are auxin producers. In the current study 98% of all isolates produced detectable levels of auxin. However, few if any studies have determined the relative auxin production capacity of a large number of auxin producing bacteria from the rhizobacterial or endorhizobacterial communities. In such studies, random isolation is necessary to remove bias associated with pre-selection of individual isolates that often occurs in studies whose prime objective is the isolation and characterization of plant growth promoters or pathogens. More importantly, no studies have yet to relate auxin production capacity of randomly selected isolates to overall biomass productivity.

Further support for a negative relationship comes from a reanalysis of data from (Hussain & Hasnain, 2011) which was generated using a completely different approach. The authors determined the auxin production capacity of 12 selected isolates and tested them for their plant growth promotion ability by inoculating wheat plants and measuring their growth response. Our reanalysis of the data showed that plant biomass productivity was negatively correlated ($R^2 = -0.55$) with *in vitro* auxin concentrations.

Rhizosphere vs Endorhizosphere : The production of IAA by rhizobacteria have been well documented. Bacteria isolated from the endorhizosphere also have the ability to produce auxin (Gangwar & Kaur, 2009). The endorhizobacteria are more intimate with the plant and have ready access to plant produced carbon which may enhance greater bacterial cell growth and auxin production potential. The supply of nutrient inside the plant tissue is consistent and more readily available, so less competition for nutrient between individual bacteria (Jhala, Shelat, Vyas, & Panpatte, 2015) which means they have less growth restrictions and are likely to produce higher auxin levels than those in the endorhizosphere. While the rhizosphere outside the roots has less intimate access to carbon and possibly less cell growth rates and auxin production potential. Water and nutrient supply is inconsistent for rhizobacteria, so high level of competition among bacteria resulting in lower growth rates could select for lower auxin production potential.

Large and Small Colony: This report is the first to examine the relationship between large and small colony bacteria with respect to auxin production capacity. Colony size may reflect the rate of initial growth and adaptation for a given media. Large colonies are those who after 4 days were the first to adapt to the new nutrient environment in the 0.1X TSA

plate. Small colonies are those that were less adapted and slower growing than the large colony isolates. However, from our experiment, large colony bacteria had a significantly higher average auxin production capacity than small colony bacteria in the rhizosphere and endorhizosphere (Table 1 and 2). Previously we found a strong correlation between auxin production potential and cell growth. Bacteria that are growing rapidly also have a greater potential to produce auxin, so large colony bacteria may also produce more auxin than slow growers. Auxin may actually be a growth regulating factor in the bacterial community, just as auxin influences cell growth in multicellular plants.

Soil Type: Soil type has no significant effect on auxin production capacity by bacteria. However, the Teller soil had a higher numerical value than the Easpur soil. The Teller soil is a sandy soil with a pH 6.7 while the Easpur loam soil is a loam soil which has a higher pH 8.0. Soil pH might be a factor in the higher auxin production capacity in the Teller fine sandy loam soil. pH is the measurement of activity of hydrogen ion concentration. Bacteria typically thrive well in soils with a near neutral pH are called neutrophils. Microbes such as bacteria are often very sensitive to hydrogen ion concentration in their environment. In fact, pH is one of the most important environmental variables that help to distinguish bacterial community composition (Antoniou et al., 1990). Higher pH may retard general bacterial growth which would result in lower auxin production.

SUMMARY

Of the 4320 bacteria isolated, 98% had the capacity to produce auxin, but the correlation with wheat biomass accumulation was mostly negative and insignificant. This presents evidence that auxin production by itself is not associated with wheat growth. The relationship between biomass and auxin production capacity in both the rhizosphere and endorhizosphere of the Teller soil was for the most part negative and not significant except for the endorhizosphere large colony bacteria which had a significant negative relationship. Also, the relationship between auxin production capacity and biomass from the rhizosphere of both Teller and Easpur soils were not significant with a non-significant negative relationship between auxin production capacity by bacteria and wheat biomass. Bacteria isolated from the endorhizosphere of wheat plant have greater auxin production capacity than those from the rhizosphere. Also, the large colony bacteria have the ability to produce higher amount of auxin than small colony bacteria isolated from wheat plant. Auxin production capacity increases and is highly correlated to cell growth suggesting a growth coordinating function for auxin within the bacteria community. There was no significant difference in the average production of auxin by isolates obtained from the rhizospheres of plants grown in the Teller and Easpur soil. However, the Teller soil had a higher numerical auxin production value as compared to the Easpur soil which could be accounted for by the difference in pH and its effect on bacteria growth and auxin production.

REFERENCES

- Ahmad, F., Ahmad, I., & Khan, M. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological research*, 163(2), 173-181.
- Al-idani, M. S. H. (2011). *Characterization of Auxin Producing Rhizobacteria in Wheat Plant Differing In Shoot Biomass*. (Master of Science), Oklahoma State University.
- Ali, B., Sabri, A., Ljung, K., & Hasnain, S. (2009). Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. *Letters in applied microbiology*, 48(5), 542-547.
- Antoniou, P., Hamilton, J., Koopman, B., Jain, R., Holloway, B., Lyberatos, G., & Svoronos, S. A. (1990). Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. *Water Research*, 24(1), 97-101.
- Bally, R., Thomas-Bauzon, D., Heulin, T., Balandreau, J., Richard, C., & Ley, J. D. (1983). Determination of the most frequent N₂-fixing bacteria in a rice rhizosphere. *Canadian Journal of Microbiology*, 29(8), 881-887.
- Cassán, F., Perrig, D., Sgro, V., Masciarelli, O., Penna, C., & Luna, V. (2009). *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). *European Journal of Soil Biology*, 45(1), 28-35.
- Chalupowicz, L., Barash, I., Panijel, M., Sessa, G., & Manulis-Sasson, S. (2009). Regulatory interactions between quorum-sensing, auxin, cytokinin, and the Hrp regulon in relation to gall formation and epiphytic fitness of *Pantoea agglomerans* pv. *gypsophilae*. *Molecular Plant-Microbe Interactions*, 22(7), 849-856.
- Costacurta, A., & Vanderleyden, J. (1995). Synthesis of Phytohormones by Plant-Associated Bacteria. *Critical Reviews in Microbiology*, 21(1), 1-18.
- Díaz-Zorita, M., & Fernández-Canigia, M. V. (2009). Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. *European Journal of Soil Biology*, 45(1), 3-11.
- Fett, W. F., Osman, S. F., & Dunn, M. F. (1987). Auxin Production by Plant-Pathogenic Pseudomonads and Xanthomonads. *Applied and Environmental Microbiology*, 53(8), 1839-1845.
- Gangwar, M., & Kaur, G. (2009). Isolation and characterization of endophytic bacteria from endorhizosphere of sugarcane and ryegrass. *Internet J Microbiol*, 7, 139-144.
- Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil. *Science*, 309(5739), 1387-1390.
- Glickmann, E., & Dessaux, Y. (1995). A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology*, 61(2), 793-796.
- Glickmann, E., Gardan, L., Jacquet, S., Hussain, S., Elasmri, M., Petit, A., & Dessaux, Y. (1998). Auxin Production Is a Common Feature of Most Pathovars of

- Pseudomonas syringae*. *Molecular Plant-Microbe Interactions*, 11(2), 156-162. doi:
- Hartmann, A., Lemanceau, P., & Prosser, J. I. (2008). Multitrophic interactions in the rhizosphere 船 Rhizosphere microbiology: at the interface of many disciplines and expertises. *FEMS microbiology ecology*, 65(2), 179-179.
- Hiltner, L. (1904). Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründung und Brache. *Arbeiten der Deutschen Landwirtschaftlichen Gesellschaft*, 98, 59-78.
- Hussain, A., & Hasnain, S. (2011). Interactions of bacterial cytokinins and IAA in the rhizosphere may alter phytostimulatory efficiency of rhizobacteria. *World Journal of Microbiology and Biotechnology*, 27(11), 2645-2654.
- Jhala, Y., Shelat, H., Vyas, R., & Panpatte, D. (2015). Biodiversity of Endorhizospheric Plant Growth Promoting Bacteria. *Journal of Biofertilizers & Biopesticides*, 6(1), 1.
- Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, 96(3), 473-480.
- Khalid, M., Zahir, Z. A., Waseem, A., & Arshad, M. (1999). Azotobacter and L-tryptophan application for improving wheat yield. *Pakistan Journal of Biological Science*, 2, 739-742.
- Kloeppe, J. W., Rodríguez-Kábana, R., Zehnder, A. W., Murphy, J. F., Sikora, E., & Fernández, C. (1999). Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. *Australasian Plant Pathology*, 28(1), 21-26.
- Klopper, J. W., Schippers, B., & Bakker, P. (1992). Proposed elimination of the term endorhizosphere. *Phytopathology*, 82(7), 726-727.
- Leveau, J. H. J., & Lindow, S. E. (2005). Utilization of the Plant Hormone Indole-3-Acetic Acid for Growth by *Pseudomonas putida* Strain 1290. *Applied and Environmental Microbiology*, 71(5), 2365-2371.
- Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S., & Castro, P. M. L. (2010). Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biology and Biochemistry*, 42(8), 1229-1235.
- Mole, B. M., Baltrus, D. A., Dangl, J. L., & Grant, S. R. (2007). Global virulence regulation networks in phytopathogenic bacteria. *Trends in Microbiology*, 15(8), 363-371.
- Monine, M. I., Posner, R. G., Savage, P. B., Faeder, J. R., & Hlavacek, W. S. (2010). Modeling Multivalent Ligand-Receptor Interactions with Steric Constraints on Configurations of Cell-Surface Receptor Aggregates. *Biophysical Journal*, 98(1), 48-56.
- Morris, R. O. (1995). Genes specifying auxin and cytokinin biosynthesis in prokaryotes *Plant Hormones* (pp. 318-339): Springer.
- Mullins, G. L., & Sikora, F. J. (1994). Effect of soil pH on the requirement for water-soluble phosphorus in triple superphosphate fertilizers. *Fertilizer research*, 40(3), 207-214.

- Nakbanpote, W., Panitlurtumpai, N., Sangdee, A., Sakulpone, N., Sirisom, P., & Pimthong, A. (2013). Salt-tolerant and plant growth-promoting bacteria isolated from Zn/Cd contaminated soil: identification and effect on rice under saline conditions. *Journal of Plant Interactions*, 9(1), 379-387.
- Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., . . . Jones, J. D. G. (2006). A Plant miRNA Contributes to Antibacterial Resistance by Repressing Auxin Signaling. *Science*, 312(5772), 436-439.
- O'Donnell, P. J., Schmelz, E. A., Moussatche, P., Lund, S. T., Jones, J. B., & Klee, H. J. (2003). Susceptible to intolerance—a range of hormonal actions in a susceptible Arabidopsis pathogen response. *The Plant Journal*, 33(2), 245-257.
- Patten, C. L., & Glick, B. R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*, 42(3), 207-220.
- Patten, C. L., & Glick, B. R. (2002). Role of Pseudomonas putida Indoleacetic Acid in Development of the Host Plant Root System. *Applied and Environmental Microbiology*, 68(8), 3795-3801.
- Peer, W. A., Blakeslee, J. J., Yang, H. B., & Murphy, A. S. (2011). Seven Things We Think We Know about Auxin Transport. *Molecular Plant*, 4(3), 487-504.
- Raaijmakers, J., Paulitz, T., Steinberg, C., Alabouvette, C., & Moëgne-Locozz, Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, 321(1-2), 341-361.
- Remans, R., Spaepen, S., & Vanderleyden, J. (2006). Auxin signaling in plant defense. *Science (New York, NY)*, 313(5784), 171.
- Robert-Seilaniantz, A., Grant, M., & Jones, J. D. (2011). Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology*, 49, 317-343.
- Sánchez-Rodríguez, C., Rubio-Somoza, I., Sibout, R., & Persson, S. (2010). Phytohormones and the cell wall in Arabidopsis during seedling growth. *Trends in Plant Science*, 15(5), 291-301.
- Spaepen, S., & Vanderleyden, J. (2011). Auxin and Plant-Microbe Interactions. *Cold Spring Harbor Perspectives in Biology*, 3(4).
- Spaepen, S., Vanderleyden, J., & Remans, R. (2007). *Indole-3-acetic acid in microbial and microorganism-plant signaling* (Vol. 31).
- Travis, S. W., Harsh Pal, B., Grotewold, E., & Vivanco, J. M. (2003). Root Exudation and Rhizosphere Biology. *Plant Physiology*, 132(1), 44-51.
- van Loon, L. C. (2007). Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology*, 119(3), 243-254.
- Yang, S., Zhang, Q., Guo, J., Charkowski, A. O., Glick, B. R., Ibekwe, A. M., . . . Yang, C.-H. (2007). Global Effect of Indole-3-Acetic Acid Biosynthesis on Multiple Virulence Factors of Erwinia chrysanthemi 3937. *Applied and Environmental Microbiology*, 73(4), 1079-1088.
- Zúñiga, A., Poupin, M. J., Donoso, R., Ledger, T., Guiliani, N., Gutiérrez, R. A., & González, B. (2013). Quorum Sensing and Indole-3-Acetic Acid Degradation Play a Role in Colonization and Plant Growth Promotion of Arabidopsis thaliana by Burkholderia phytofirmans PsJN. *Molecular Plant-Microbe Interactions*, 26(5), 546-553.

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Candidate for the Degree of

Master of Science

Thesis: THE RELATIONSHIP BETWEEN AUXIN PRODUCTION CAPACITY BY RHIZOBACTERIA AND WHEAT BIOMASS

Major Field: Plant and Soil Science

Biographical:

Education:

Completed the requirements for Master of Science in Plant and Soil Science at Oklahoma State University, Stillwater, Oklahoma in December, 2015.

Completed the requirements for Master of Science in Crop Science at Njala University, Sierra Leone, 2012.

Completed the requirements for Bachelor of Science in Crop Protection at Njala University, Sierra Leone, 2011.

Experience:

1. The skills acquired extracting rhizosphere bacteria, endorhizosphere bacteria, culturing bacteria, extracting bacteria DNA, extracting plant DNA auxin assay and PCR.
2. Workshop on student leadership forum – 2015
3. Training on the impact of climate change and desertification on agriculture and food security in Burkina Faso – 2013
4. Training on strengthening capacity in innovation platform and value chain approach - 2013
5. Training on basic principles and practices of Biotechnology– 2012
6. Training on sustainable agriculture, environment and the community - 2012
7. Research Officer at Sierra Leone Agricultural Research Institute - 2011

Professional Memberships:

2014 – present. Member of American Society of Agronomy

2014 – present. Member of Honor Society of American

2011- present. Member of Sierra Leone Agricultural Researcher's