

Evaluation of Plant Growth Promotion by Wild-type *Bacillus amyloliquefaciens* FZB42 on tomato

I. Experiment conducted by: Camilo Ramírez

II. Objectives

- Determine the potential for tomato plant growth promotion by *Bacillus amyloliquefaciens* FZB42.
- Optimize the inoculation procedure with this strain.

III. Materials and Methods

a. Effect of different application methods and inoculum concentrations on growth promotion of tomato.

A 4 X 3 factorial experiment was utilized. Factors were concentration of inocula (10^4 , 10^6 , 10^8 , 10^{10} cfu/ml) and application methods (seed, transplant and combined). Tomato seeds (hybrid 'Juliet'), soil-less plant growth medium ("promix®") and bacterial strain *B. amyloliquefaciens* FZB42 (commercial product containing 2.97×10^{11} cfu/ml of the strain determined by Most Probable Number) were used. Seeds were sown in 32-cell trays. Bacterial suspensions were prepared at 10^4 , 10^6 , 10^8 , 10^{10} cfu.mL⁻¹ from the commercial product. The seed treatment was done by inoculating 1 mL of each concentration onto the seed. Four weeks later these seedlings were transplanted into six inches pots. The transplant treatment was applied at this time for those seedlings that were not previously inoculated by adding 80 mL of each concentration into the pot. The combined treatment included both application methods, which are seed and transplant. The control received 1 mL of distilled water per seed at the seeding and 80 mL per pot at transplant. Pots were kept on the bench in the Auburn University Greenhouse, watered with a hand sprayer and fertilized once a week as recommended for tomato. Plants were harvested three weeks after transplant and fresh, dry shoot and root weight recorded. Each treatment had eight replications.

b. Bacterial Population Counting and Tomato Root Architecture.

For the inoculum concentration 10^8 cfu.mL⁻¹ (the selection of this concentration was based on results of the previous section), total bacterial and *Bacillus* spp. populations were measured. For both populations 10 g of root and soil-less mix were taken from each plant. Samples were shaken in a sterilized flask with 100mL of SDW for 20 minutes. From each flask 1 mL of the suspension was

taken by pipette for serial dilution and total population was determined by plate counting onto 50% Trypticase Soy Agar. *Bacillus* population was measured by treating the serial dilution in a water bath at 80 °C for 13 minutes before plating. Four plants per treatment were used for population counting and root architecture analysis (using Winrhizo© software). Root architecture included the measurement of root length, surface area, volume, diameter, and number of tips.

All data recorded was analyzed by using SAS program (SAS inc. 1997).

IV. Results and Discussion

a. Effect of different application methods and inoculum concentrations on growth promotion of tomato.

For the factor 'application method', the main component analysis showed significant differences at $p=0.05$ on the variables dry shoot weight, fresh and dry root weight. For these variables the best application method was at transplant. For the factor 'concentration', only dry root weight was significantly different.

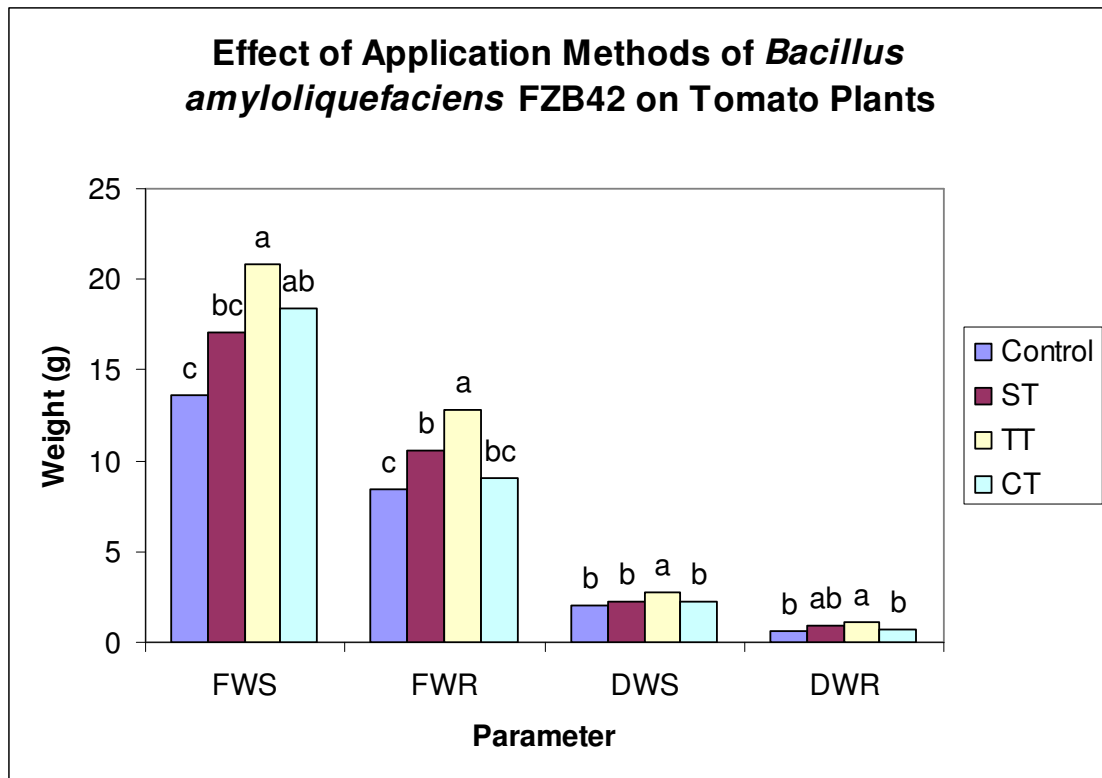
The results for experiment comparing the four concentrations of *B. amyloliquefaciens* FZB42 with 3 different application methods are provided in Table 1 below. Mean dry shoot weight and mean dry root weight were significantly greater in treatments using 10^8 cfu.mL⁻¹ inoculated at transplant.

Table 1. Response of tomato plants to different application methods and concentrations of *Bacillus amyloliquefaciens* FZB42. Values followed by the same letter are not significantly different at $P=0.05$.

Treatment	Dry Shoot Weight (g)			Dry Root Weight (g)		
	Seed	Transplant	Combined	Seed	Transplant	Combined
10E4	2.2 bcde	2.3 bc	2.0 cde	0.76 bcde	0.78 bcd	0.56 e
10E6	2.3 bcd	2.4 ab	2.0 de	0.73 bcde	0.69 cde	0.65 cde
10E8	2.3 bcd	2.7 a	2.2 bcd	0.93 ab	1.09 a	0.73 bcde
10E10	2.2 bcd	2.4 b	1.9 e	0.93 ab	0.83 bc	0.59 de
Control	2.0 cde	2.0 cde	2.0 cde	0.66 cde	0.66 cde	0.66 cde

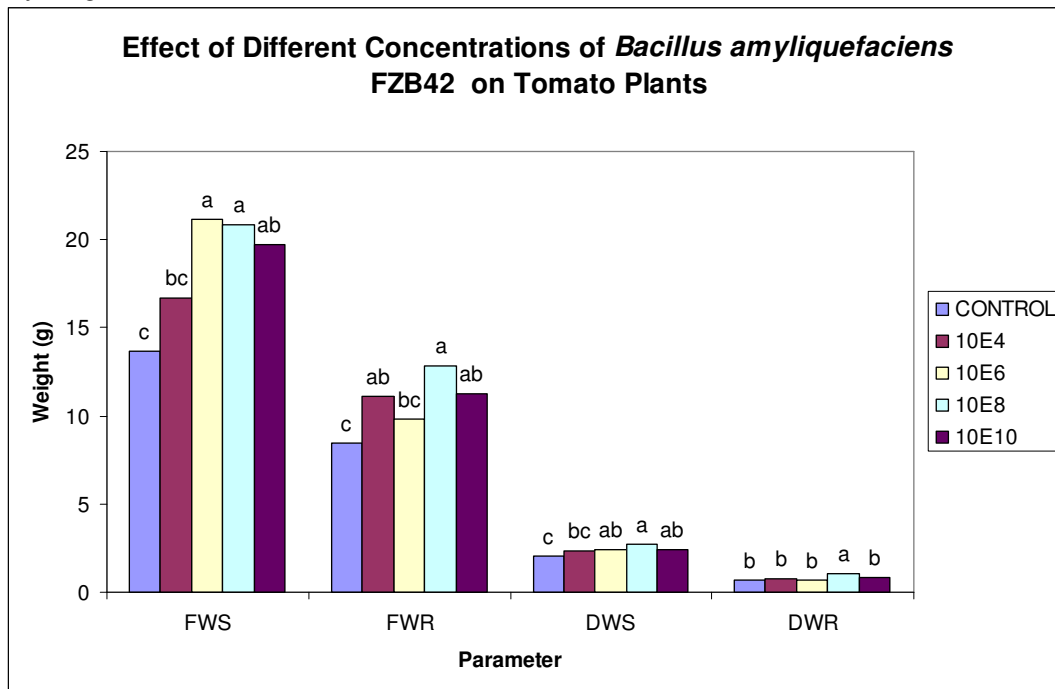
From these results we concluded that 10^8 cfu.mL⁻¹ is the best inoculation concentration. For this dosage (10^8 cfu.mL⁻¹) we analyzed application strategies by comparing growth parameters. The results illustrated in Figure 1. below show that the greatest fresh shoot weight, dry shoot weight, fresh root weight and dry root weight were obtained when inocula were applied at transplant.

Fig 1. Effect of three application methods on tomato by plant growth promoting rhizobacteria strain *B. amyloliquefaciens* FZB42 three weeks after transplanting for inoculum concentration of 10^8 cfu/mL. ST indicates seed inoculation, TT transplanting application, CT combined application. FWS indicates fresh shoot weight, FWR fresh shoot weight, DWS dry shoot weight and DWR dry root weight. Different letters indicate significant differences among fresh or dry weight at $P= 0.05$.



To verify the results which indicated 10^8 cfu.mL⁻¹ being the best application rate, growth parameters were compared for the most productive application strategy: at transplant. In Figure 2., below, inoculum concentrations are compared for each growth parameter at 10^8 cfu.mL⁻¹. For each parameter the mean values increase directly with concentration until reaching 10^8 cfu.mL⁻¹. However, after this optimal concentration, increases in dosage result in negative effects on plant growth.

Fig 2. Effect on tomato of four different concentrations of plant growth promoting rhizobacteria strain *B. amyloliquefaciens* FZB42 three weeks after transplanting for transplant treatment. FWS indicates fresh weight shoot, FWS indicates fresh shoot weight, FWR fresh shoot weight, DWR dry shoot weight and DWR dry root weight. Different letters indicate significant differences among fresh or dry weight at P= 0.05.



b. Bacterial Population Counting and Tomato Root Architecture.

Root architecture components were measured to compare each application method at 10^8 cfu.mL⁻¹. The results are provided in Table 2. Root surface area was the only parameter determined to be significantly greater when inoculants were applied at transplant.

Table 2. Effect on tomato root architecture of plant growth promoting rhizobacteria strain *B. amyloliquefaciens* FZB42 in soil-less medium for the inoculum concentration 10^8 cfu/ml. Different letters indicates statistically significant difference (P=0.05) among treatments.

Treatment	Length (cm)	Surface Area (cm ²)	Volume (cm ³)	Diameter (mm)	Number of tips
Seed inoculation	520.5a	430.16 b	29.13a	2.7 a	1619.5 a
Transplant inoculation	673 a	626.83 a	48.32a	3.1 a	2297.3 a
Combined inoculation	548.7a	480.66 b	40.54a	3.1 a	1822.5 a
Control	520.7a	503.77 b	33.62a	2.8 a	1633.8 a

LSD _{0.05}	189.07	101.59	15.92	0.9	807.64
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In Table 3 total log bacterial population and log *Bacillus* population are given for inoculation concentration 10⁸ cfu.mL⁻¹. No significant difference was found for total bacterial population, suggesting a buffer effect from plant-soil-bacterial interactions in all treatments. However, in the *Bacillus* population count, transplant inoculation and combined inoculation were significantly greater than the control and seed inoculation. We observed in plates streaked from rhizobacteria the same morphological colony features found in plates of the commercial product. In addition, the bacterial colonies found in the control were morphologically different from those rhizobacteria colonies of the commercial product. In addition, the calculated values of the proportion of *Bacillus* population for the transplant and combined inoculations were greater than those of seed inoculation and the control, signifying that a higher percentage of the total bacterial population (in these application strategies) consists of *Bacillus* spp., i.e., those applied for growth promotion. While *Bacillus* populations were found to be greater in transplant and combined inoculations, only transplant application yielded the highest growth parameters demonstrated earlier. Perhaps this disparity can be explained by the negative growth effects experienced by plants at inoculum concentrations greater than 10⁸ cfu.mL⁻¹. Samples collected prior to those taken for this experiment may have yielded higher and more accurate values for combined inoculation bacterial populations.

Table 3. Effect on total bacterial and *Bacillus* population of plant growth promoting rhizobacteria strain *B. amyloliquefaciens* FZB42 in soil-less medium for the inoculum concentration 10⁸cfu/ml. Different letters indicates statistically significant difference (P=0.05) among treatments.

Treatment	Total Population log cfu/g	<i>Bacillus</i> population log cfu/g	Ratio
Seed inoculation	7.25 a	5.32 b	1.27 b
Transplant inoculation	7.35 a	5.91 a	3.99 a
Combined inoculation	7.34 a	5.92 a	4.38 a
Control	7.15 a	5.02 b	0.77 b
LSD _{0.05}	0.244	0.319	2.07

In summary, growth is promoted by *Bacillus amyloliquefasciens* FZB42. Factors affecting optimal growth include application time and inoculation concentration. For this particular strain and under our experimental conditions, 10⁸ cfu.mL⁻¹ and transplant application yielded maximum growth promotion for the parameters measured in this study. For each PGPR strain this type of study should be conducted to determine the optimum application time and inoculation concentration. During the first 3 weeks of the study a phosphorus deficiency occurred due to an error in the greenhouse fertilizer system. In addition, this

study was conducted during the months of October and November, when ambient temperature is below typical greenhouse-study temperatures. These below-normal temperatures can also contribute to phosphorus deficiency in tomato. In conclusion, we observed a correlation between the growth parameters and *Bacillus* population which indicates a change in community structure, as opposed to a change in community number.