Scientific Bulletin. Series F. Biotechnologies, Vol. XX, 2016
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

PHYTOHORMONE–LIKE PRODUCING *BACILLUS* INCREASE TOMATO SEEDLINGS QUALITY

Oana-Alina SICUIA, Sorina DINU, Florica CONSTANTINESCU

Research and Development Institute for Plant Protection,
8 Ion Ionescu de la Brad Blvd., Bucharest, Romania

Corresponding author email: sicuia_oana@yahoo.com

Abstract

Plant growth promoting activity is one of the attributes when searching for beneficial strains of bacteria. Our study aimed to present the potential of Bacillus sp. 83.2s and B. subtilis Bce2 to increase plant growth and vigour. The study is based on phytohormone - like evaluation in the selected Bacillus strains using classic biochemical analysis. Likewise, the plant beneficial effect was also evaluated by growth promotion studies on tomato seedlings, were biometric parameters and chlorophyll content index (CCI) were analyzed. The data have been processed into the following indicators: indole-3-acetic acid in bacterial cultures, and emergence, seedling height and vigour, shoot and root dry weight and CCI in tomatoes seedlings. Both bacterial strains produce high amounts of IAA phytohormones, from 10.4 - 13.8 µg/ml, in normal growth medium, up to 16.3 - 16.6 µg/ml, when 5 mM of tryptophan is added in the medium as auxin precursor. Moreover, B. subtilis Bce2 seed treatment, increased emergence index and seedlings vigour compared to the untreated control and exceeded the commercial growth regulator (Vimpel 77%) in terms of emergence percent, seedlings dry weight, and chlorophyll content index.

Key words*: Bacillus, tomato seedlings.*

INTRODUCTION

Tomato is a main crop in the greenhouse production system, cultivated with good results also in open fields. The main technology for growing tomatoes in our country is from seedlings. Regarding these aspects the quality of planting material is an important issue in tomatoes production. Seedlings quality is even more important in organic production. For healthy and vigorous planting material there are several studies recommending beneficial microorganisms inoculation.

Plant beneficial microorganisms are natural environmental microbes that could be exploited in terms of organic agriculture and biodiversity maintenance. The *Bacillus* genus includes a large number of plant growth-promoting rhizobacteria (PGPR). *Bacillus* bio-based products are highly appreciated in organic farming, not only for their plant protection and growth promotion abilities, but also for their endospore forming ability that gives stable, long lasting, easy to formulate and preserve inoculums.

Among the complex mechanisms involved in plant growth promotion, bacteria can produce phytohormone-like compounds and lytic enzymes, and can increase mineral uptake, nutrients availability and reconvert agricultural wastes (Goswami et al., 2016).

The aim of the preset study is to analyze plant growth promoting ability and phytohormone production in two biocontrol strains of *Bacillus* spp. Plant beneficial activity was evaluated in tomato seedlings maintained in growth chamber conditions.

MATERIALS AND METHODS

Bacterial inoculum

Two bacterial strains were previously selected for their plant beneficial activity (Sicuia et al., 2015; Dinu et al., 2015). Bacterial strains used in this study were Romanian isolates *Bacillus* sp.83.2s and *B. subtilis* Bce2. These bacteria were routinely grown on Luria Bertani medium at 28°C. For bacterial cell suspension, 48h old broth cultures were centrifuged at 3750rpm, for 15minutes, at 10ºC and the pellet was resuspended in phosphate buffered saline (PBS) up to 10^8cfu/ml. The concentration was estimated spectro-photometric at 600nm wavelength when the optical density reached the absorbance of 1.

IAA synthesis ability

The auxin synthesis in bacterial cultures was evaluated in Luria Bertani broth (LB) and LB supplemented with tryptophan in concentration of 2.5M, and 5M, respectively. Cultures were inoculated with 10% bacterial suspension of 10^8cfu/ml. After 24h of cultivation at 28°C, and 150rpm stirring, the supernatant was harvested by centrifugation and analyzed for auxin quantification. Two ml of each supernatant was treated with 3 drops of o-phosphoric acid, and 4ml Salkowski reagent ($FeCl_3$-$HClO_4$). The homogenate was incubated for 25 min at room temperature. Subsequently IAA was spectrophotometric quantified at 530nm. The results were correlated with a standard curve containing 10 ÷ 100mg IAA/ml.

***In situ* evaluation of plant-growth-promoting activity on tomatoes seedlings**

The study was performed on tomato *Lycopersicon esculentum* Heinz 2274 cultivar. Seeds were surface disinfected, with 70% ethanol, by 3 minutes immersion and subsequently, with sodium hypochlorite 4% by 15 min. immersion, than rinsed ten times with sterile distilled water. For plant growth promotion study, seeds were treated with bacterial cell suspension 10^8cfu/ml PBS, supplemented with 2‰ carboxy-methyl cellulose to ensure the adhesion of the inoculum to the seeds tegument. As positive control a commercial plant growth regulator, Vimpel 77%, was applied as seed treatment and as soil fertigation 20ml/plant, two weeks after seed germination, both treatments in 0.05% concentration. For the negative control, untreated, seeds were immersed in sterile distilled water.

Alveolar trays with peat mixture were seeded with one seed/cell, having 7cm diameter and 8cm depth. Thirty seeds were sown for each experimental variant.

Plants were maintained for five weeks in SANYO MLR-351H growth chamber under a 16 h daylight period. Light intensity, around trays, was approximately 14000 lx. The air temperature was set at 25°C during the day and 16°C during the night. Relative humidity was constant at 70%, and the soil moisture was maintained with tap water.

Plant growth and vigour parameters

The emergence rate (EP) was evaluated one week after sowing.

$$EP\% = \frac{\text{Emerged seedlings}}{\text{Sown seeds}} \times 100$$

Emergence index (EI) was calculated by adapting the formula mentioned by Geetha et al. (2014) for the germination index:

$$EI = \frac{EP\% \text{ in treatment}}{EP\% \text{ in control}}$$

Four weeks after plantlets emerged shoots were measured in order to determine their length. At the end of the experiment, plants were gently removed from the substrate and roots were washed with tap water.

Fresh and dry weight of the shoot and root were taken. The dry weight was determined after drying the plant material at 105°C for at least 3h. To determine the seedling vigour index (SVI) we used two calculation methods, by adapting the formulas presented by Adebisi et al. (2010) and Alirezaie Noghondar and Azizi (2013) for SVI – 1 and SVI – 11, respectively.

$$SVI-1 = \frac{\text{Seedling lenght} \times EP\%}{100}$$

$$SVI-11 = \frac{\text{Seedling dry weight} \times EP\%}{100}$$

Chlorophyll content index

Relative chlorophyll content was estimated with a Chlorophyll Content Meter (CCM-200plus, Opti-Sciences) in intact leaf samples, using a non-destructive method. The Chlorophyll Content Index (CCI) values are determined by the absorbance ratio at two wave lengths. One wavelength falls within the chlorophyll absorbance range while the other serves to compensate for mechanical differences such as tissue thickness (www.apogeeinstruments.com).

$$CCI = \frac{\text{Transmittance\% at 931nm}}{\text{Transmittance\% at 653nm}}$$

The CCI values are proportional with the chlorophyll content in the sample.

RESULTS AND DISCUSSIONS

IAA synthesis in *Bacillus* cultures

Bacteria cultures of *Bacillus* sp.83.2s and *B.subtilis* Bce2 were analyzed for IAA quantification after 24hours of growth in simply LB broth and LB supplemented with tryptophanas auxin precursor. In this respect the optical densities determined at 530 nm were related to the IAA calibration curve with known auxin content.

IAA quantification showed that *Bacillus* sp. 83.2s strain is a better auxin producer in LB broth than *B.subtilis* Bce2 strain. However, when the growth medium was supplemented with tryptophan, Bce2 revealed to produce more IAA than the other strain tested (Table 1).

Table 1. IAA amount in tested *Bacillus* cultures

Experimental variants	IAA (µg/ml)		
	LB broth	LB & 2.5 mM tryptophan	LB & 5 mM tryptophan
Bacillus sp. 83.2s	13.8	15.6	16.3
B. subtilis Bce2	10.4	16.1	16.6

As the results shown, the two analyzed strains are producing higher amounts of auxin(Figure 1)compared to other plant beneficial strains of *Bacillus* sp. mentioned in the literature. Acuña et al. (2011) mentioned that *Bacillus* sp. MQH-19 strain produced only 3 to 6 µg IAA/ml. In similar growth conditions, *Bacillus* sp. Q3 strain produced 3.76 to 10.62 µg IAA/ml (Starovic et al., 2013). However, in yeast malt dextrose broth with or without tryptophan, the amount of IAA would significantly increase 5 to 8 fold (Mohite, 2013).

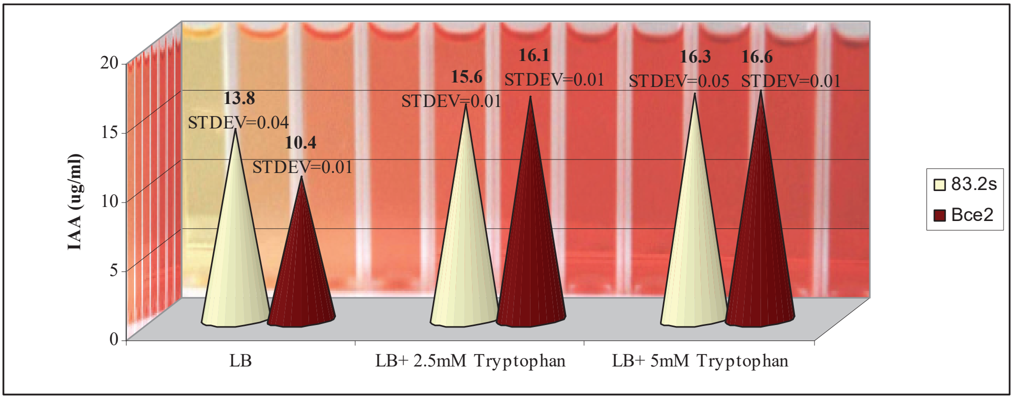

Figure 1. Comparative IAA production in *Bacillus* sp.83.2s and *B. subtilis* Bce2 cultures (after 24h of incubation)

Plant growth promotion

Bacterial seed treatment promoted the tomatoes seedling growth (Table 2). All seeds germinated and seedlings emerged when Bce2 treatment was applied. The other bacterial treatment, 83.2s, provided the same emergence rate (90%) as in the positive control, treated with Vimpel. While in the untreated control the emergence percentage was 83.3%.

Table 2. Tomato seedlings growth promotion with bio-based treatments

Specifications	**EP%**	EI	**Seedling length (cm)**	**Shoot dry weight (g)**	**Root dry weight (g)**	**Seedling vigour DW/SH**	SVI-1	SVI-11
Bacillus sp. 83.2s	90%	1.08	8.7	2.38	0.28	0.31	7.83	2.39
Bacillus. subtilis Bce2	**100%**	**1.2**	9.3	**2.77**	**0.31**	**0.33**	9.30	**3.08**
Vimpel 77% Positive control	90%	1.08	**11.0**	2.23	0.25	0.23	**9.90**	2.32
Untreated control	83.3%	-	8.6	1.92	0.20	0.25	7.16	1.77

The Vimpel treatment induced seedling elongation in a higher rate than the other treatments (Figure 2). However, excessive stem elongation is considered a drawback (Figure 3). Comparing seedlings height (SH) with their dry weight (DW), it can be noticed that Bce2 treatment enhanced seedlings growth promotion with a better ratio (as DW/SH).

Regarding seedling weight the best results were registered in the bacterial treatment with Bce2 strain (3.08g), followed by 83.2s (2.66g) and Vimpel (2.48g). Both bacteria treatments increased plantlets biomass (as DW), compared to the untreated control, as well as if comparing with the positive control. Bce2 increased seedlings DW with 45.3%, 83.2 with 25.5% and Vimpel with 17% compared to the untreated control that developed 2.12 g of DW. Also compared to the positive control (Vimpel) the two bacterial treatments, Bce2 and 83.2s, led to an increased DW content, with 24.2% and 7.3%, respectively (Figure 2).

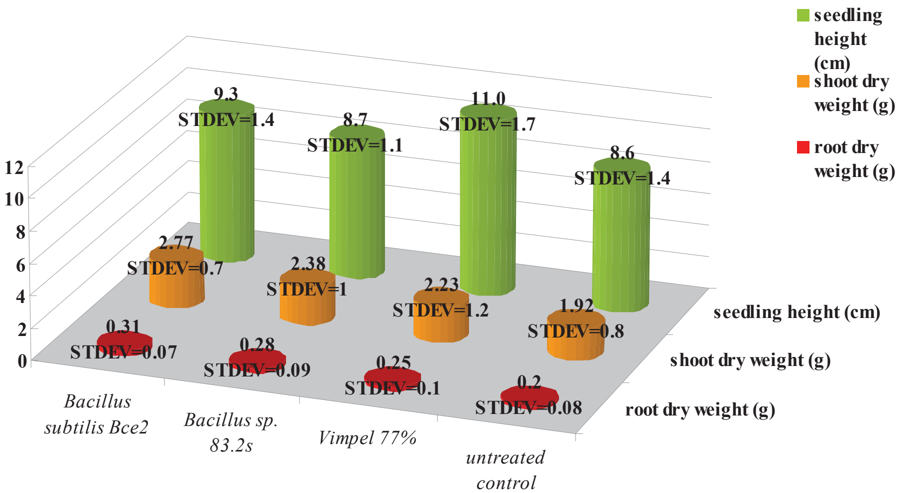

Figure 2. The influence of *Bacillus* treatments on vegetative growth of tomato seedlings

Figure 3. Tomato seedlings with bio-based treatments

The seedling vigour index (SVI), as calculated with Adebisi et al. (2010) and Alirezaie Noghondar and Azizi (2013) formulas, reveal *B.subtilis* Bce2 and Vimpel as competitive treatments for growth promoters.

Relative chlorophyll content

The highest CCI values were registered in the Bce2 bacterial treatment followed by 83.2s treatment (Table 3).

Table 3. Relative chlorophyll content in tomato leafs during seedling growth

Specifications	Weeks after emerging							
	1st		2nd		3rd		4th	
	CCI	STDEV	**CCI**	STDEV	**CCI**	STDEV	**CCI**	STDEV
Bacillus sp. 83.2s	**13.2**	4.26	**25.13**	6.38	**28.69**	5.79	**34.76**	5.5
B. subtilis Bce2	**14.44**	3.86	**26.18**	6.08	**28.89**	5.26	**37.12**	3.99
Vimpel 77% Positive control	**11.51**	2.74	**23.3**	4.68	**24.91**	4.02	**27**	4.78
Untreated control	**11.32**	1.47	**21.15**	5.18	**26.71**	4.28	**33.54**	6.7

A positive correlation was observed between seedlings vigour (as DW/SH) and their relative chlorophyll content. This aspect is more evident in the experimental variant treated with Vimpel, especially after the fertigation, when the chlorophyll content was significantly slowed. The lowest CCI values, registered after the second treatment with Vimpel, could be due to a growth stimulation that leaded to an increased seedling elongation without promoting seedling vigour (correlation of Tables 2 and 3).

CONCLUSIONS

The two strains proposed in the present study, *Bacillus* sp. 83.2s and *B. subtilis* Bce2, revealed plant growth promotion activity *in vitro* and *in vivo* in seedlings trials. Both strains produced IAA phytohormone. In normal culture conditions a higher amount of IAA was produced by 83.2s strain (13.8μg/ml). However, when auxin precursor was added into the growth medium Bce2 strains showed a higher capacity of tryptophan conversion that lead to an IAA production of 16.6 μg/ml.

Bacillus subtilis Bce2, applied as seed treatment, increased the germination percent and seedlings vigour compared to the untreated control and exceeded the commercial growth regulator (Vimpel 77%) in terms of germination, shoot and root dry weight, and chlorophyll content index.

ACKNOWLEDGEMENTS

This work was supported by the RDIPP NUCLEU Project PN 02-01 financed by the Ministry of National Education and Scientific Research.

REFERENCES

Acuña J.J., Jorquera M.A., Martínez O.A., Menezes-Blackburn D., Fernández M.T., Marschner P., Greiner R., Mora M.L., 2011. Indole acetic acid and phytase activity produced by rhizosphere bacilli as affected by pH and metals. Journal of Soil Science and Plant Nutrition, 11 (3): 1-12.

Adebisi M.A., Okelola F.S., Alake C.O., Ayo-Vaughan M.A., Ajala M.O., 2010. Interrelationship between seed vigour traits and field performance in new rice for africa (Nerica) genotypes (*Oryza sativa* L.). Journal of Agricultural Science and Environment, 10(2):15-24.

Alirezaie Noghondar M., Azizi M., 2013. Seed harvesting time afects seedling emergence, vigour and growth: Case study of *Rumex turcomanicus* Czerep. (*Polygonaceae*). Notulae Scientia Biologicae, 5(2):244-248.

Dinu S., Sicuia O.A., Constantinescu F., 2015. The influence of several abiotic factors on some bacterial strains of phytosanitary use. Romanian Journal of Plant Protection, 8:1-6.

Geetha K., Chaitanya K., Bhadraiah B., 2014. Isolation and characterization of PGPR isolates from rhizosphere soils of Green gram in Warangal district of Telangana. Int J Pharm Bio Sci, 5(4): (B) 153 – 163.

Goswami D., Thakker J.N., Dhandhukia P.C., 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture, 2(1):1127500.

Mohite B., 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of Soil Science and Plant Nutrition, 13(3): 638-649.

Sicuia O. A., Grosu I., Constantinescu F., Voaideş C., Cornea C. P. 2015. Enzymatic and genetic variability in *Bacillus* spp. strains with plant beneficial qualities. AgroLife Scientific Journal, 4(2): 124-131.

Starovic M., Josic D., Pavlovic S., Drazic S., Postic D., Popovic T., Stojanovic S., 2013. The effect of IAA producing *Bacillus* sp. Q3 strain on marshmallow seed germination. Bulgarian Journal of Agricultural Science, 19 (3): 572-577.