

***Bacillus subtilis* as Growth Promotor in Hydroponically Grown Tomatoes under Saline Conditions**

Markus Voitke¹, Helmut Junge², Wilfried H. Schnitzler¹ Chair of Vegetable Science - Quality of Vegetal Foodstuff

Center of Life Science, Technische Universität München, Freising, Germany

²FZB Biotechnik GmbH, Berlin, Germany

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Abstract

The gram-positive bacterium *Bacillus subtilis* is known to positively influence plant growth, vitality, and the ability of the plant to cope with pathogens often resulting in higher yield. These beneficial effects are documented in field crops, where it has been ascribed to abiotic stress release. However, little is known about the mechanism by which *B. subtilis* interacts with the plant. Therefore, we tested a specifically selected strain of *B. subtilis* (FZB24) for its ability to help hydroponically grown tomato plants to withstand salinity stress. The basic nutrient solution was 2.5 mS/cm EC with the addition of 2 and 4 mS/cm NaCl EC plus 5 mmol CaCl in all treatments to reach 5.4 and 7.4 mS/cm EC. In addition, the high salinity level (EC NaCl 4) was inoculated with the plant growth promoting bacteria *B. subtilis* in the root zone. Plant growth parameters including element concentrations in plant tissues were examined on tomato plants grown under 3 salinity regimes in an environmentally controlled greenhouse. Marketable yield was significantly reduced in the high salinity treatment as compared to the control without NaCl. In addition, the high salinity treatment with *B. subtilis* had even lower yield despite improved vegetative plant growth. The element composition/concentration in the leaves is discussed according to salinity levels.

INTRODUCTION

Plant-growth-promoting-rhizobacteria (PGPR) are free-living microorganisms having beneficial effects on plants by colonizing their roots. The concept of PGPR has gained acceptance over the last decade, and several possible mechanisms have been proposed for their effects. They include such effects as the suppression of plant pathogenetic diseases (Smith et al., 1999), the exclusion of pathogens from the roots by competition (Dekkers et al., 1998), enhancing the release of limited available nutrients from the soil matrix (Nautiyal et al., 2000, Richardson et al., 2001b, Idriss et al., 2002) and the release of plant-growth regulating substances such as IAA – indole-3-acetic acid (Steenhoudt and Vanderleyden, 2000).

The identification, selection and application of suitable beneficial microorganisms can increase the options to deal with growing problems (Kilian et al., 2000), and additionally, can be environmentally sound.

The gram-positive bacterium *Bacillus subtilis* is one of those (Fig. 1), mainly used in the USA as a seed dressing over the past ten years, applied to more than 2 million ha, mainly in potato and maize (Backman et al., 1994). It is known to positively influence plant vitality and the ability of the plant to cope with abiotic stressing conditions such as drought and salinity (Bochow et al., 2001; Junge, Bochow, pers. com.).

However, it has been shown that the organism performs by colonization of the plant roots, the longest demonstrated in artificial and sterilized substrates (Batinic et al. 1998, Grosch et al., 1996). This is often regarded as a consequence of its weak competitive ability as compared to many other microorganisms in the soil during plant growth (Grosch et al., 1999, Junge and Bochow, pers. com.).

However, these attributes and the fact that little is known about the mechanisms by which *B. subtilis* interacts with plants, especially as an abiotic stress mediator (Idriss et

al., 2002, Junge, Bochow, pers. communication) make the organism suitable to study for plant growth and nutrient uptake in relation to stressing conditions in a more controlled system such as soilless cultivation. We assumed that its beneficial effects may be the highest and best demonstrated under such an experimental setup.

MATERIALS AND METHODS

Plant Material and Culture Conditions

Tomatoes (cultivar Douglas, Fa Juliwa, Germany) were grown in a closed soilless hydroponical system from March 21 (transplanting) – July 14, 2004, in a climatically controlled greenhouse (320 m², at the experimental station Freising, Germany). As a substrate perlite (0-6 mm) was used in 10 l pots. Plant density was 2 plants/pot, 3 plants/m² (0.3 x 1.2 m), 4 replicates (n = 14). Plants were grown until June 15 and then topped (above the ninth truss).

Salinity levels, fertigation and watering: EC 3.4 (nutrient solution plus 5mM CaCl₂ without NaCl (control)); EC 5.4 (nutrient solution plus 5mM CaCl₂ plus 17mM NaCl), EC 7.4 (nutrient solution plus 5mM CaCl₂ plus 34 mM NaCl), EC 7.4 (nutrient solution plus 5mM plus 34 mM NaCl) plus *Bacillus subtilis*. Salinization of the nutrient solution started 20th of April without any adaptation.

Nutrient solution: Macronutrients (mmol) 10 NO₃, 1 NH₄, 6.5 K, 1.25 P, 1.25 Mg, 1.5 S, 1 Cl. Micronutrients (μmol) 15 Fe, 10 Mn, 20 B, 0.75 Cu, 4 Zn, 0.5 Mo. Every two weeks nutrient solution was analysed for adaptation of the stock solution to guarantee an optimal nutrient supply to the plants (Programm Substrafeed, Fa Hydro Agri, The Netherlands). Sodium chloride and calcium chloride in the nutrient solution were adjusted 2-times a week and adapted to requested levels (Test kids, Fa Merk, Germany). Watering (drip irrigation, 4 drippers per pod) was at fixed intervals, every 20 min, 5 min (8l /h) in a closed system.

Inoculation of *Bacillus subtilis*, strain FZB24[®]WG (FZB, Biotechnik GmbH, Germany), took place at the 4 leaf stage of the juvenile plants and was applied as spore solution (0,02% w/w) 7 times over a week (ones each day, ~50 ml/plant). Additionally, after transplanting into the greenhouse, the spore solution was added once more.

Evaluation Parameters

Collected plant data were leaf area, fresh and dry weight, fruit yield, fruit size and element analysis of the leaves (C, N, Ca, Mg, K, Na and P).

Statistical Analysis

Values are depicted as means if not otherwise stated. Analysis of variance was conducted using the program Statistica (Version 5.5, Tulsa, USA, 1999). F-Test and the Least Significant Difference (LSD) were used for comparison between treatments. Means are supposed to be significantly different at the 5 % probability level.

RESULTS AND DISCUSSION

Growth parameters: *Bacillus subtilis* influenced plant growth manifold. After the onset of salinization (23.04.), tomato leaf development had a distinct response to the inoculation with *B. subtilis*, and less to the salinity itself. Plants in the other treatments reacted differently: the higher the salinity the smaller the leaves, both in length and width. In contrast, the high salinity treatment plus *B. subtilis* had, notwithstanding salinity, the largest leaves. This tendency was stable until the end of the experiment on July 15, when the *Bacillus* inoculated plants exhibited highest leaf area per plant (Fig. 2). Additionally, plants inoculated with *B. subtilis* yielded the highest number of leaves as compared to the other variants, where no differences occurred. Analysis of the Dw/Fw-ratio indicated that in the presence of *B. subtilis* the water content of the leaves was significantly increased (Fig. 2).

Fruit harvest: However, leaf size was not paralleled by a similar increase neither in

fruit productivity nor in fruit quality (Fig. 3). Fruit yield (kg) in relation to salinity levels and *B. subtilis* inoculation were significantly different, increasing salinity decreased the sum of marketable fruit and increased the percentage of non-marketable fruit. The number of large fruit (> 45 mm) was significantly higher in the control treatment. The higher the salinity the smaller was the fruit (Fig. 3, right side). Comparing high salinity treatment with and without *B. subtilis*, inoculated plants had more reduced yield (20%) and a double fraction of non-marketable fruit. More than 90% percent of deficient fruit had symptoms of BER (blossom end rot). Fruit production was lowered over the whole harvesting period under higher salinity levels and the lowest in the *Bacillus* treatment.

Water and nutrient uptake, leaf element composition: Water uptake in the *Bacillus* treatment appeared to be unaffected or less affected after salinization started. This is demonstrated by drastically increased concentrations of Na (Cl) in mature leaf tissues (youngest fully developed leaves) during the time course directly after the onset of salinization (Fig. 4). The same is true for the Na-concentrations of weekly pruned shoots and leaves, where Na-concentrations were increased 3 times in the *B. subtilis* treatment. The differences still were detectable at the end of the trial, but less pronounced, indicating an induced adaptation of the plants. Therefore, a function of *B. subtilis* could be the maintenance of water influx after osmotic stress that occurred in the root environment and/or a faster osmotic adjustment at the plant tissue level balancing the raised osmotic pressure in the medium. The concentrations of some other macro-nutrients in mature leaf tissues were also positively influenced through the presence and activity of the microorganism in the rhizosphere of the tomato plants, predominantly N and Mg (Table 1), but also other elements such as K (negatively), Ca (positively) appeared to be more affected by the salinity level itself. This reinforces the observation of a more vigorous growth of the *B. subtilis* inoculated plants, favoring vegetative over reproductive growth.

The beneficial effects of *B. subtilis* on plant growth and vitality under stressing conditions as reported from field sites proved to be more complex and less predictable than expected after these preliminary results in an artificial soilless growing system. Nevertheless, the significant influences of the bacterium on plants is obvious from these preliminary observations. Especially, favoring fruit production over vegetative growth should be addressed in future experiments, if *B. subtilis* will have a future for soilless growing practice under stress conditions. In this regard, the physiological interactions between plant, microorganism, and stressors should be investigated.

ACKNOWLEDGEMENTS

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Tables

Table 1: Element concentrations (g/100g dw) in youngest fully developed leaves before salinization, 20 d after and at the end of the cultivation period (compounded samples n = 30).

	Before salinization (April 8)				20 d after salinization (May 8)				90 d after salinization (July 15)			
	C	5.4	7.4	7.4 + b.s.	C	5.4	7.4	7.4 + b.s.	C	5.4	7.4	7.4 + b.s.
Ca	2.20	2.45	1.70	2.12	2.05	2.19	1.78	2.64	3.72	4.04	4.01	4.78
Mg	1.16	1.21	0.91	1.03	0.45	0.42	0.37	0.53	0.66	0.57	0.80	0.93
K	4.57	5.16	4.27	5.01	5.64	5.60	5.48	5.41	4.52	4.00	3.72	3.41
P	0.63	0.75	0.59	0.67	0.86	0.83	0.98	0.70	0.64	0.66	0.57	0.64
C	37.4	36.2	38.6	37.5	38.0	38.2	38.1	34.3	34.4	35.9	35.5	34.5
N	4.41	4.91	4.43	4.54	4.59	4.95	4.54	5.79	4.48	4.03	4.44	5.79
Na	0.11	0.14	0.10	0.12	0.05	0.42	0.37	1.50	0.08	0.65	0.87	1.00

Figures

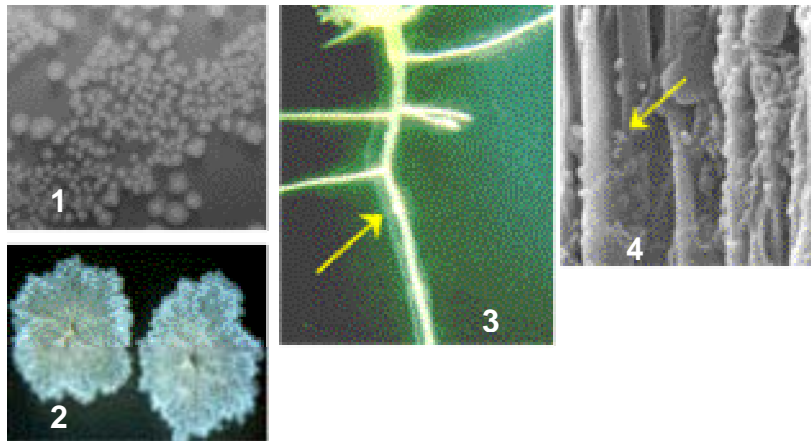


Fig. 1. (1-4). *Bacillus subtilis* colonies (1+2, petri-dish cultures); 3 colonized root hairs (Dr. Thomzik, Bayer AG) and 4 endozoospores on roots (Dr. Schmedeknecht, Humboldt University Berlin).

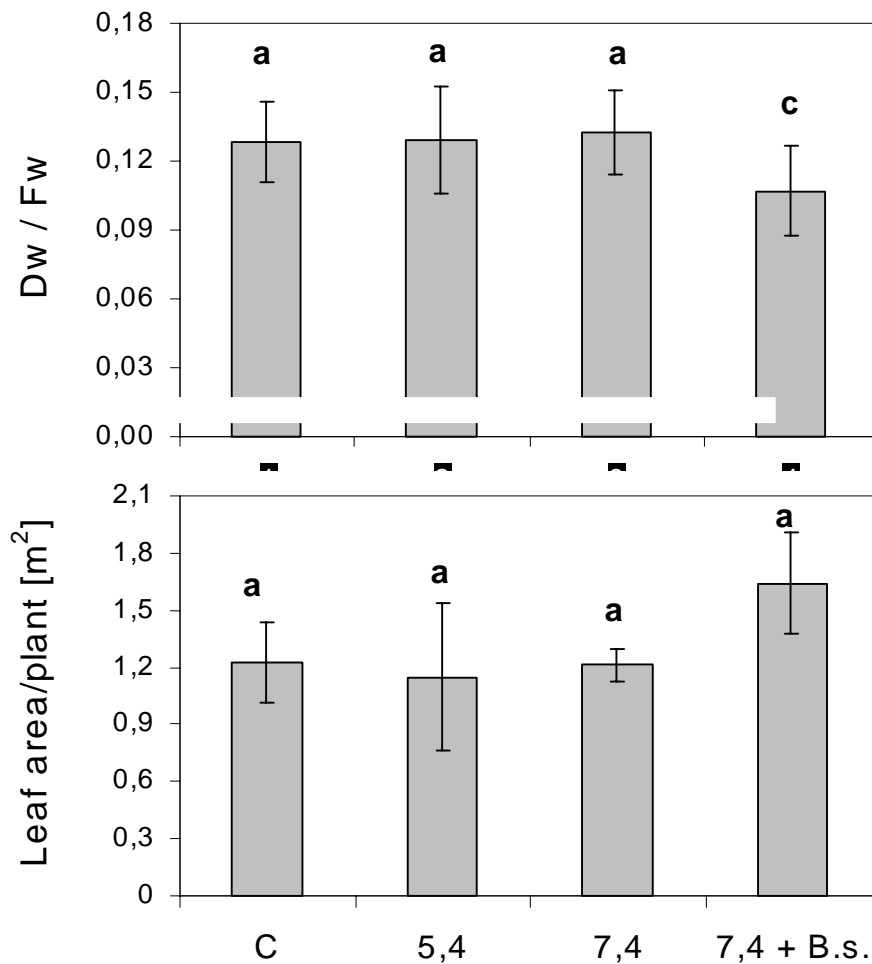


Fig.2. Plant growth parameters for plant leaf area and dw/fw-ratio in relation to salinity levels and the inoculation with *B. subtilis* (n = 4. LSD < 0.05).

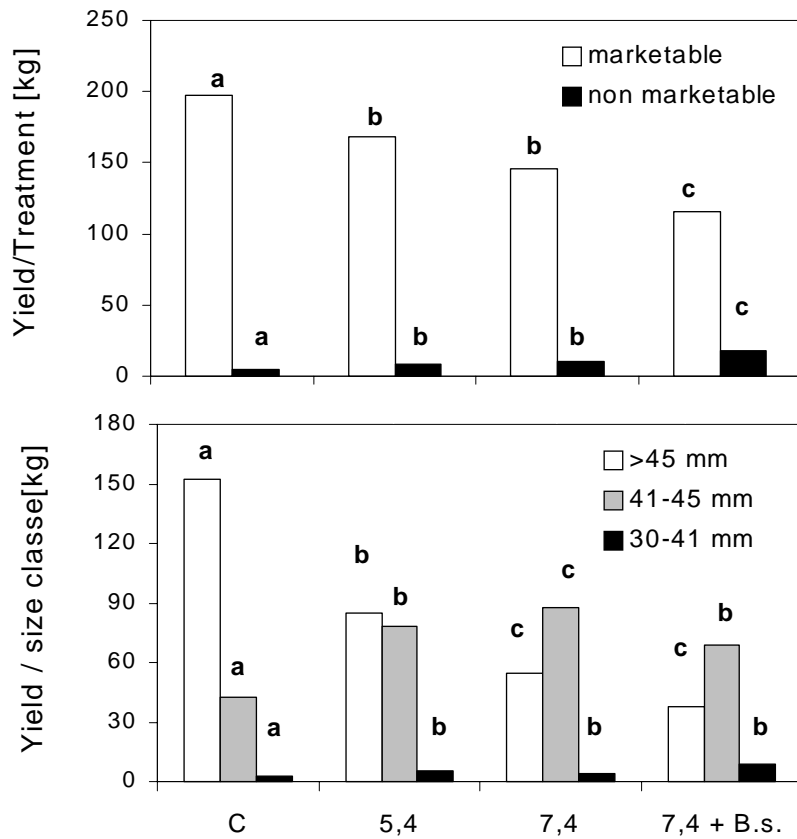


Fig. 3. Yield of marketable fruit and fruit size in relation to salinity levels and *B. subtilis* inoculation (n = 4. LSD < 0.05).

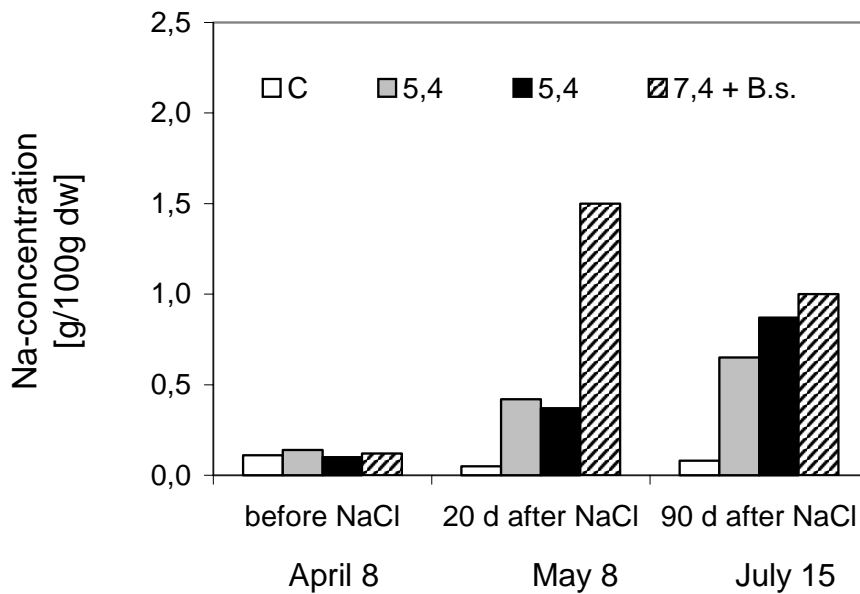


Fig. 4. Sodium concentrations in youngest fully developed leaves of the tomato plants over the cultivation period (before (April 8). shortly after (May 8) and at trial end (July 15) in relation to salinity levels and *B. subtilis* inoculation (n = 30, compounded samples).