

Effects of nutrition and *Bacillus amyloliquefaciens* on tomato (*Solanum lycopersicum* L.) growing in perlite

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria that, as their name suggests, promote plant growth. However, they can also be of help in the biological control of plant diseases. This study reports the effects of two different commercially available strains of *Bacillus amyloliquefaciens* (FZB24 and FZB42) on tomato production in open and closed systems in the presence of different amounts of nutrients. Three factors were tested: (1) the type of nutrition system (open or closed), (2) the concentration of the nutrient solution (full or half strength), and (3) the PGPR applied (either *B. amyloliquefaciens* FZB24 or *B. amyloliquefaciens* FZB42, or a no-PGPR control). Perlite was used as the growth medium. Variables related to water use efficiency, yield and fruit quality were assessed. The use of half strength nutrient solution was sufficient for full growth in the open system in both spring and autumn seasons. However, the same strength nutrient solution was associated with reduced yields in the closed system during the autumn season. The application of either strain of *B. amyloliquefaciens* increased the yield of the tomato plants by 8-9% in the open system in the spring, whereas they had an adverse effect on yield in the closed system under half strength nutrient solution conditions during the autumn.

Additional key words: closed system, nutrient concentrations, open system, plant growth-promoting rhizobacteria, soilless culture.

Resumen

Efectos de la nutrición y de *Bacillus amyloliquefaciens* en tomate (*Solanum lycopersicum* L.) cultivado en perlita

Las rizobacterias promotoras del crecimiento de las plantas (PGPR) son bacterias que viven libres y, como su nombre indica, promueven el crecimiento de las plantas. Sin embargo, también pueden ser de ayuda en el control biológico de las enfermedades de las plantas. En este estudio se investigaron los efectos de dos cepas comerciales de *Bacillus amyloliquefaciens* (FZB24 y FZB42) sobre la producción de tomate en sistemas abiertos y cerrados, en presencia de diferentes cantidades de nutrientes. Los tratamientos fueron: (1) tipo de sistema de nutrición (abierto o cerrado), (2) concentración de nutrientes (solución nutritiva completa o incompleta), (3) la PGPR aplicada (*B. amyloliquefaciens* FZB24, *B. amyloliquefaciens* FZB42, o un control sin PGPR). Se utilizó perlita como medio de crecimiento. Se evaluaron las variables relacionadas con la eficiencia del uso del agua, producción de tomate y calidad del fruto. La solución de nutrientes menos concentrada fue suficiente para un pleno crecimiento en el sistema abierto en ambas estaciones (primavera y otoño). Sin embargo, la misma solución de nutrientes disminuyó en otoño la producción en el sistema cerrado. La aplicación de ambas cepas de *B. amyloliquefaciens* aumentó la producción de las plantas de tomate un 8-9% en el sistema abierto en primavera, mientras que tuvo un efecto adverso sobre la producción en otoño en el sistema cerrado con la solución menos concentrada.

Palabras clave adicionales: concentración de nutrientes, cultivo sin suelo, rizobacterias promotoras del crecimiento de las plantas, sistema abierto, sistema cerrado.

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Introduction

Increasing awareness of environmental problems during the late 1980s and early 1990s led to closed, soilless growth systems—with their more efficient use of water and fertilizers and their reduced pollution of ground and surface water—gaining in importance (Van Os, 2000; Schnitzler, 2004). However, the use of closed hydroponic systems on the commercial scale is associated with problems of crop nutrition management and the proliferation of root pathogens (Savvas, 2002). Recently, interest has grown in the use of beneficial microorganisms in soilless culture to induce plant resistance to biotic and abiotic stress factors, and to increase plant growth and yield (Böhme, 1999; Armstrong, 2001; Ehret *et al.*, 2001; Alsanius and Gertsson, 2004; Alsanius *et al.*, 2004; Koohakan *et al.*, 2004; Schnitzler, 2004; Van Os and Alsanius, 2004; Woitke and Schnitzler, 2005; Deniel *et al.*, 2006).

Plant growth-promoting rhizobacteria (PGPR) can enhance plant growth capacity by increasing seed emergence, plant weight and yield. PGPR increase the growth of a number of important crops, with some strains inducing systemic resistance to fungi, bacteria, viruses, and in some cases nematodes (Reddy *et al.*, 2000; Kloepper *et al.*, 2004a). Most of the strains used belong to species of *Pseudomonas* or *Bacillus* (Ehret *et al.*, 2001). A number of commercially available PGPR-based products are available in different countries, most of which contain strains of *Bacillus* spp. (Kloepper *et al.*, 2004b). The aim of this study was to determine the effects of two commercially available *Bacillus amyloliquefaciens* strains on tomato (*Solanum lycopersicum* L.) production in open and closed systems under different nutrient conditions. Since temperature, light intensity, day length, and relative humidity, etc., also have marked effects on plant nutrition via the increase or reduction of nutrient uptake and by changing the distribution of nutrients within the plant (Resh, 1991; Adams, 1993), experiments were performed in both the autumn and spring seasons.

Material and Methods

Plant material and growing conditions

The tomato plants used in these trials were all of the Durinta cultivar. Seedlings were produced in a commer-

cial nursery and transferred to the experimental greenhouse at the five true-leaf stage. Planting density was 3.48 plants m⁻² (plant spacings 1.15 × 0.25 m, 9 plants plot⁻¹). Plants were grown in 6 L pots containing perlite under short term production system conditions during autumn (September 3, 2004–February 7, 2005) and spring (March 4–July 8, 2005).

Treatments

The experimental design used was that of split-split plots with 3 replicates. The treatments involved the modification of the following variables: (1) the nutrition system (open or closed), (2) nutrient solution concentration (full or half strength nutrient solution), and (3) the PGPR applied [*B. amyloliquefaciens* FZB24, *B. amyloliquefaciens* FZB42, or no-PGPR treatment (control)]. *Bacillus amyloliquefaciens* FZB24 and FZB42 were provided by FZB Biotechnik GmbH, Germany. Inoculation was performed before sowing in the nursery and after transplanting to the experimental greenhouse, according to the manufacturer's recommendations. The seeds were immersed in 0.1% spore solution for 10 min before sowing, and a 0.02% spore solution was applied to the plants (20 mL per plant) after transplanting. In the no-PGPR treatment, distilled water was used instead of spore solution.

Complete nutrient solution was used to cover the water and nutrient requirements of the plants. The chemical composition of this solution was (mg L⁻¹): N 210 (240), P 40, K 250 (300), Ca 150, Mg 50, Fe 2, Mn 0.75, B 0.4, Zn 0.50, Cu 0.10 and Mo 0.05 (Day, 1991). The N and K concentrations in brackets were used after fruit setting at the third truss. With the half strength nutrient solution, macroelements were applied at half of the concentrations given above, while microelements were provided at the same concentrations. The electrical conductivity (EC) and pH of the nutrient solutions were recorded daily. EC values changed according to the treatments; pH was maintained between 6.0 and 6.5 by adding nitric acid. The timing of irrigation was based on an indoor integrated solar radiation level of 1.0 MJ m⁻². The amount of nutrient solution was adjusted according to the ratio of drain water/applied volume (drainage ratio maintained at 25–30%).

In the open system the plants were fed from two different tanks containing full or half strength nutrient solution; the drained solution from each treatment was collected in separate tanks and the volumes recorded. In the closed system, each treatment had its own tank and the original volume was maintained by adding fresh water and stock solutions daily. The stock solution was added in agreement with the volume of water added. The nutrient solution was completely changed if the EC exceeded 3.5 mS cm^{-1} .

Variables measured

— Water Use Efficiency (WUE): this was determined (kg m^{-3}) for the marketable fresh yield. WUE values were not subjected to statistical analysis since the drained water from each treatment was collected into the same tank.

— Yield: total and marketable yield was recorded as harvested fruit weight and number of fruits. Fruits with a diameter of $< 3.5 \text{ cm}$ or with physiological disorders, e.g., blossom-end rot, cracking, etc., were deemed unmarketable.

— Fruit quality: fruits sampled at the third truss were analysed to determine fruit firmness, total soluble solids (TSS), titratable acidity (TA) and the vitamin C content. Fruit firmness was determined as the displacement (mm) of the fruit in relation to pressure applied (Shewfelt, 2000). Fruit juice was extracted using a kitchen juicer and filtered through qualitative paper. TSS was measured as a percentage using a digital refractometer (Euromex RD 645, The Netherlands). Titratable acidity was determined by titration with 0.1 N NaOH until pH 8.1, and expressed as $\text{mval } 100 \text{ mL}^{-1}$. The vitamin

C content was determined as $\text{mg per } 100 \text{ mL}$ of fruit juice according to Pearson (1970).

Statistical analysis

The yield and fruit quality data were subjected to ANOVA. Means were compared using Fisher's protected least significant difference (LSD) test. Significance was set at $P \leq 0.05$.

Results

Water use efficiency

In the autumn, the WUE was 41.5% higher in the closed system (42.6 kg m^{-3}) than in the open system (30.1 kg m^{-3}). In fact, the WUE increased by 78.6% with the full nutrient concentration in the closed system (43.4 kg m^{-3}) compared to the open system (24.3 kg m^{-3}), but only 16.4% with the half nutrient concentrations (35.9 kg m^{-3} in the open system, 41.8 kg m^{-3} in the closed system). The average WUEs in the FZB24, FZB42 and control treatments were 34.8 , 37.0 and 37.3 kg m^{-3} respectively (Fig. 1A).

In the spring season, and with full strength nutrient solution, a 17.9% higher WUE was recorded in the closed system (29.7 kg m^{-3}) compared to the open system (25.2 kg m^{-3}). The WUE was 19.0% higher in the closed system with the half strength nutrient solution (25.8 kg m^{-3} in the open system, 30.7 kg m^{-3} in the closed system). The average WUEs for the FZB24, FZB42 and control treatments were 27.7 , 28.6 and 27.3 kg m^{-3} respectively (Fig. 1B).

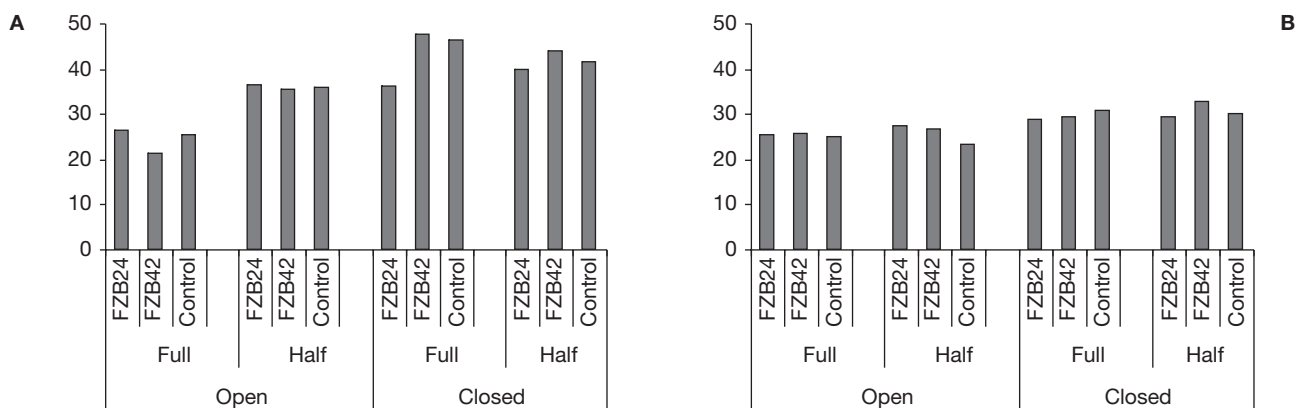


Figure 1. Water use efficiency (kg m^{-3}) in autumn (A) and spring (B).

Yield

In the autumn, the interaction *nutrition system* $\times$ *concentration of nutrient solution* $\times$ *PGPR* had a significant effect on total and marketable fruit weight and total number of fruits. *Bacillus amyloliquefaciens* was associated with a smaller yield in the closed system under the lower nutrient concentration conditions. Compared to the full strength nutrient solution, the half strength nutrient solution was associated with a smaller yield in the closed system (Table 1).

In the spring season, the nutrition system had a significant effect on the total and marketable yield and

on marketable fruit number. The closed system returned lower mean yields than the open system (13.0 kg m⁻² compared to 15.5 kg m⁻²). The interaction *nutrition system* $\times$ *PGPR* also had a significant effect on total and marketable yield. The *Bacillus* treatments (FZB24 and FZB42) increased the total and marketable yield compared to the control (14.7 kg m⁻²) in the open system [9% with FZB24 (16.0 kg m⁻²) and 8% with FZB42 (15.9 kg m⁻²)]. No significant differences were seen between *Bacillus* and control treatments in the closed system (12.9 kg m⁻² for FZB24, 12.8 kg m⁻² for FZB42, and 13.3 kg m⁻² for the control treatment) (Table 1).

Table 1. Effect of the treatments on yield

Treatments	Autumn				Spring			
	Fruit weight (kg m ⁻²)		Fruit number (no. m ⁻²)		Fruit weight (kg m ⁻²)		Fruit number (no. m ⁻²)	
	Total	Marketable	Total	Marketable	Total	Marketable	Total	Marketable
<i>Open</i>								
Full:								
— FZB24	13.5	13.4	139.3	128.9	15.7	15.4	165.8	158.1
— FZB42	12.5	12.3	131.0	117.0	15.9	15.7	192.4	186.6
— Control	12.9	12.8	141.8	131.0	15.3	15.2	165.0	162.0
Half:								
— FZB24	13.8	13.7	138.6	126.4	16.2	16.1	173.6	169.0
— FZB42	13.6	13.3	141.4	127.1	15.8	15.6	171.8	161.0
— Control	13.6	13.4	135.5	124.1	14.0	13.8	163.2	152.3
<i>Closed</i>								
Full:								
— FZB24	12.8	12.5 b	137.3	127.0	12.6	12.4	165.8	157.1
— FZB42	13.2	13.1 ab	141.3	131.7	13.2	12.7	168.5	155.6
— Control	14.1	13.9 a	141.0	132.8	13.6	13.3	155.1	149.1
Half:								
— FZB24	9.3 b	9.1 b	110.7 ab	98.1	13.2	13.1	148.9	143.2
— FZB42	9.6 b	9.6 b	98.2 b	92.9	12.3	12.1	152.0	143.8
— Control	12.4 a	12.2 a	121.8 a	112.5	13.0	12.8	149.4	141.8
F significance								
A	ns	ns	ns	ns	*	*	ns	*
B	*	*	*	*	ns	ns	ns	ns
C	*	*	ns	ns	ns	ns	ns	ns
A*B	*	*	*	*	ns	ns	ns	ns
A*C	*	*	ns	ns	*	**	ns	ns
B*C	ns	ns	ns	ns	ns	ns	ns	ns
A*B*C	*	*	*	ns	ns	ns	ns	ns

A: nutrition systems. B: concentrations of nutrient solution. C: PGPR. ns: not significant. * $P < 0.05$. ** $P < 0.01$. Different letters for values in the same subplot in each column denote significant differences ($P < 0.05$).

Fruit quality

In the autumn, the concentration of the nutrient solutions had a significant effect on the TA and vitamin C content. The interaction *nutrition system* $\times$ *concentration of nutrient solution* also had a significant effect (Table 2). The TA increased with the full strength nutrient solution compared to the half strength solution, an effect more obvious in the closed system. The vitamin C content of the fruits was higher with the half strength solution in the open system; no significant differences in this respect were recorded between the solution strengths in the closed system.

In the spring, the nutrition system had a significant effect on the vitamin C content of the fruits (lower in the open system compared to the closed system). The nutrient concentration had a significant effect on fruit firmness, TA, and the TSS of the fruit juice; the values of these variables were higher with the full strength nutrient solution. PGPR had significant effect on TA (higher with *B. amyloliquefaciens* FZB24) (Table 2).

Discussion

The results show that the WUE was higher in the closed than in the open system. Increased WUEs in

Table 2. Effect of the treatments on fruit quality

Treatments	Autumn				Spring			
	Firmness (mm)	TA (mval 100 mL ⁻¹)	TSS (%)	Vitamin C (mg 100 mL ⁻¹)	Firmness (mm)	TA (mval 100 mL ⁻¹)	TSS (%)	Vitamin C (mg 100 mL ⁻¹)
<i>Open</i>								
Full:								
— FZB24	1.75	5.66	3.60	11.85	1.60	6.68	4.33	9.96
— ZB42	1.06	5.14	3.30	11.85	1.70	6.21	4.50	10.62
— Control	1.65	5.56	3.90	10.68	1.23	6.20	4.30	9.69
Half:								
— FZB24	0.99	4.38	3.63	12.89	2.07	5.67	4.13	12.15
— FZB42	1.20	4.42	3.53	13.12	1.85	5.60	4.23	11.99
— Control	1.88	4.46	3.33	13.08	2.32	5.36	4.10	13.40
<i>Closed</i>								
Full:								
— FZB24	1.20	5.86	3.70	13.69	1.73	8.05	4.70	12.22
— FZB42	1.38	5.83	3.93	12.88	1.62	6.77	4.50	14.69
— Control	1.30	5.93	4.07	12.75	1.92	6.15	4.60	16.32
Half:								
— FZB24	1.21	4.19	3.67	13.34	1.95	6.31	4.20	15.52
— FZB42	1.17	4.35	3.67	12.93	2.17	5.69	4.33	19.43
— Control	1.03	4.15	3.53	12.68	2.32	5.22	4.13	14.25
F significance								
A	ns	ns	ns	ns	ns	ns	ns	**
B	ns	*	ns	*	*	**	**	ns
C	ns	ns	ns	ns	ns	**	ns	ns
A*B	ns	*	ns	*	ns	ns	ns	ns
A*C	ns	ns	ns	ns	ns	ns	ns	ns
B*C	ns	ns	ns	ns	ns	ns	ns	ns
A*B*C	ns	ns	ns	ns	ns	ns	ns	ns

TA: titratable acidity. TSS: total soluble solids. A: nutrition systems. B: concentrations of nutrient solution. C: PGPR. ns: not significant. * $P < 0.05$. ** $P < 0.01$.

closed systems are well documented (Vernooij, 1992; Van Os, 1995, 1999; Tuzel *et al.*, 1999, 2001, 2004; Gul *et al.*, 2006). Differences were seen between the open and closed systems with respect to yield according to the growing season; no significant differences were seen in the autumn, but the yield was higher in the open system in the spring. The smaller yield in the closed system in the spring can be explained by the negative effect of higher salinity on water uptake and plant growth under conditions of high solar radiation (Schwarz and Kuchenbuch, 1998).

The interaction *nutrition system* $\times$ *concentration of nutrient solution* had a significant effect on yield and fruit quality. In the open system, the half strength nutrient solution was sufficient for full growth in both seasons. These results agree with those reported by Adams (1993), showing that normal growth is possible when low nutrient concentrations are maintained continuously and never allowed to deplete. Morard *et al.* (2004) compared two nutrient concentrations (20.00 and 3.25 meq L⁻¹) in a tomato growing setting, and indicated that dilute solutions might be associated with higher yields than those normally used in soilless tomato cultivation culture. However, in the present work, the half strength nutrient solution was associated with a reduced yield in the closed system during the autumn. This may be the result of the depletion of some elements in the circulating solution. It is reported that nutrient concentrations in the substrate are lower in closed systems (Ferrante *et al.*, 2000). It is also well known that under poor light conditions in winter, nutrient concentrations should be increased to improve plant growth and fruit setting (Sonneveld, 2002). The fruit quality results show that the full strength nutrient solution increased the TA and TSS of the fruit juice. This supports that indicated by Cuartero and Fernández-Munoz (1999), who reported TSS and TA to increase with salinity. The full strength nutrient solution also gave rise to an increase in fruit firmness in the spring. Similarly, Hao and Papadopoulos (2004) reported that a high EC, whether constant or variable, increases the firmness of summer production fruit.

Although both formulations of *B. amyloliquefaciens* reduced the yield obtained in the closed system under lower nutrient levels during the autumn season, they gave higher yields (FZB24 9% and FZB42 8%) than the control in the open system during the spring season. In field trials with different plant species, PGPR has been found to increase yield, generally by some 10–15% (Zhang *et al.*, 2004). Studies related to PGPR in

hydroponics have mainly concentrated on their antagonism of harmful microorganisms. It is reported that pseudomonad PGPRs can increase the fresh weight of cucumber fruits by 18% (McCullagh *et al.*, 1996). One strain of *Bacillus subtilis* increased cucumber yield by 14% compared to *Pythium aphanidermatum*-inoculated controls (Utkhede *et al.*, 1999).

PGPR, applied at the sowing/transplanting stage, are often used to control harmful microorganisms in hydroponics systems and should be able to promote growth in diseased as well as healthy plants (Van Os and Alsanis, 2004). In the present study, the application of *B. amyloliquefaciens* increased the yield of the tomato plants by 8–9% in healthy conditions without nutrient stress. This increase is acceptable compared with the average yield increases reported by Zhang *et al.* (2004). However, *B. amyloliquefaciens* had an adverse effect on yield in the closed system under nutrient stress conditions in the autumn. This supports our previous report that the adverse effect of *B. subtilis* on yield in closed systems may depend on nutritional imbalances in the system (Gul *et al.*, 2006). In addition, Vavrina (1999) indicated that *Bacillus* treatments reduce the root to shoot ratio in pepper transplants, which might have a negative effect if water or nutrients become limiting.

Nowadays, environmental pollution and water shortages have a key bearing on the improvement of soilless culture. Closed systems have gained in importance, and the use of beneficial microorganisms offers very interesting new approaches for the biological control of root-infesting pathogens in these systems. However, the influence of the rhizosphere conditions on the interactions between beneficial microorganisms and plant roots should be taken into account. In the present work, the response of the tomato plants to *B. amyloliquefaciens* appeared to change according to the type of nutrition management. Further investigations are needed to understand the relationships between beneficial microorganisms and plants in soilless culture with the aim of understanding the environments most suitable for them.

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