# PLANT GROWTH PROMOTION OF TOMATO BY A BIOLOGICAL PREPARATION (LS213) AND EVALUATION FOR PROTECTION AGAINST *CUCUMBER MOSAIC VIRUS*

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## ABSTRACT

Previous work in our lab demonstrated that specific individual strains of PGPR, when applied to cucumber and tomato seeds, resulted in induced systemic protection against *Cucumber mosaic virus* (CMV) in field trials. We report here results of studies aimed at determining if similar protection against CMV could result from the use of a PGPR-based biological preparation used to enhance growth of tomato transplants. The biological preparation, termed LS213, contained industrial formulated spores of *Bacillus subtilis* GB03, *Bacillus amyloliquefaciens* IN937a and chitosan. This preparation was mixed into a soil-less media used to prepare tomato transplants according to standard industry procedures. Greenhouse trials were conducted to determine effects of the treatment on protection against CMV infection, and if infection occurred, disease severity. Results showed that treatment with LS213 significantly increased the growth of tomato transplants, irrespective of the concentrations or potting medium used, compared to the carrier and a non-treated control. At 4 weeks post-inoculation, no significant differences were observed between treatments in percentage of plants infected by CMV. Disease severity was relatively similar among treatments with the exception that some CMV infected, non-bacterized plants were stunted while none of the infected LS213-treated plants showed stunting. CMV-infected plants treated with LS213 subsequently demonstrated pronounced plant growth promotion and recovery from CMV symptoms on new growth, while controls remained stunted. Plants treated with LS213 bloomed earlier and exhibited significantly greater fruit weight than controls.

## INTRODUCTION

Most approaches for biocontrol of plant diseases and plant growth promotion have used applications of single biocontrol agents, such as plant growth promoting rhizobacteria (PGPR). This may partially account for the reported inconsistent performance by biological preparations, because a single biological agent is not likely to be active in all soil environments in which it is applied or against all pathogens that attack the host plant. The biological preparation LS213, which contains industrial formulated spores of *Bacillus subtilis* strainGB03 as a growth-promoting agent, *B. amyloliquefaciens* strain IN937a as an induced resistance agent and 2.5% chitosan as carrier, was tested for capacity to promote growth of tomato in a transplant system (Reddy *et al*., 1999; Kenney *et al*., 1999; Kloepper *et al*., 1999).

*Cucumber mosaic virus* (CMV) is one of the most important viruses affecting production of field-grown vegetables worldwide. CMV is difficult to control due to its

broad host range in excess of 800 plant species and because it is transmitted in a non-persistent manner by more than 60 species of aphids.

In previous studies under field and greenhouse conditions some PGPR strains elicited systemic protection of tomato or cucumber to CMV (Raupach *et al*. 1996; Zehnder *et al*. 2000) or under field conditions in tomato against the whitefly-transmitted geminivirus Tomato mottle virus (Murphy et al., 2000). In these studies, the PGPR were applied as individual strains without being formulated. The objective of this study was to determine if the formulated product, LS213, could also elicit plant growth promotion and ISR against CMV.

## MATERIALS AND METHODS

The initial set of experiments focused on the effects on tomato plant growth and development in response to variations in the biological treatment. The biological preparation, LS213 (described above) was mixed into various soil-less media, including Fafard's-mix, Pro-Mix, Vermiculite, and Speedling-Mix at a ratio of 1:40 (v/v) prior to seeding transplant trays with tomato (cv. Solar Set). Controls included non-treated and chitosan alone. Different concentrations of LS213 (1:20, 1:40, 1:60, 1:80, 1:100, 1:200) and 1:40 of chitosan were mixed with Pro-Mix. There were four replicated trays per treatment. Height, stem diameter, shoot fresh weight, leaflet surface area, root fresh weight were evaluated as parameters of tomato growth.

For the CMV experiment, tomato plants were grown in Pro-Mix containing LS213 at 1:40 (v/v). At two weeks after seeding, tomato plants were transplanted into 15-cm diameter pots and were inoculated mechanically with CMV three weeks later. Inoculum consisted of CMV-infected tobacco leaf tissue ground in 50 mM $KPO_4$, pH 7.5, containing 10 mM Sodium sulfite at a ratio of 1 g tissue:5ml buffer. CMV incidence was reported by determining the percentage of plants showing CMV-induced symptoms such as mosaic and distortion of foliar tissues and the stunting of plant growth.

Data were analyzed with ANOVA using JMP software followed by Student's test for least significant differences at $P$=0.05.

## RESULTS

LS213, when added to the mix before seeding, significantly increased the growth of tomato seedlings, compared to the nontreated control and the chitosan treatment alone. This growth promotion occurred at all concentrations (Table 1) and in all soil-less media tested (Table 2) compared to non-treated control. Growth of tomato plants was enhanced by increasing concentrations of LS213 from 1:200 (0.5%) to 1:20 (5%) (Table. 1) but decreased as the chitosan concentration increased from 1% to 4% (data not shown).

There was no difference in the timing of symptom appearance or percentage of plants expressing CMV-induced symptoms between treatments (Table 3). Plants expressing symptoms initially developed systemic vein-clearing followed by mosaic symptoms on younger leaves. These symptoms were followed by leaf curling with some strap-leaf symptoms characteristic of CMV infection. The primary distinction between LS213-treated plants and plants in either of the control treatments was that nontreated control plants and chitosan-treated plants became stunted (i.e., reduced plant height and

leaf size), whereas the LS213-treated plants expressed no signs of stunting. Furthermore, the LS213-treated plants tended to continue to grow with some suppression in symptom development over time (Table 4, Fig. 1).

## DISCUSSION

The use of PGPR-mediated resistance and plant growth promotion requires a delivery system which is practical on a large scale. Using the biological preparation, LS213, for preparation of transplants offers such a practical delivery system. Based on the results reported here with plant growth promotion, LS213 could be used to generate tomato transplants 1-2 weeks earlier than the typical methods used in the vegetable transplant industry, which would reduce costs of production.

CMV is a persistent threat to production of many crops, particularly tomato. Because young plants are typically more severely affected by CMV infection than old plants, plant growth promotion of tomato at the seedling stage may provide a means to shorten this window of vulnerability. Treatment of tomato plants with LS213 did not protect plants from infection with CMV, relative to controls, but this treatment did significantly reduce CMV-induced symptom severity and yield losses. The enhanced growth of LS213-treated tomato plants appeared to result in a form of tolerance to the infection rather than resistance.

**Table 1.** Effect of different concentrations of LS213 on tomato growth 4 weeks after seeding

| Treatments[1] | Vigor[2] | Height (cm)[3] | Stem diameter (mm)[4] | Shoot fresh weight (g)[5] | Leaflet surface area ($cm^2$)[6] | Root fresh weight (g)[7] |
|---|---|---|---|---|---|---|
| LS213 1:20 | 4.0 * | 14.54 * | 2.91 * | 2.39 * | 5.33 * | 0.49 * |
| LS213 1:40 | 3.6 * | 14.62 * | 2.84 * | 2.23 * | 5.80 * | 0.99 * |
| LS213 1:60 | 3.3 * | 13.50 * | 2.92 * | 1.97 * | 5.64 * | 0.72 * |
| LS213 1:80 | 3.1 * | 12.95 * | 2.68 * | 1.59 * | 5.22 | 0.60 * |
| LS213 1:100 | 2.5 * | 12.04 * | 2.81 * | 1.61 * | 3.66 | 0.44 |
| LS213 1:200 | 2.3 | 10.67 | 2.28 | 1.18 | 4.43 | 0.40 |
| Chitosan 1:40 | 2.9 * | 12.04 * | 2.96 * | 1.92 * | 6.10 * | 0.18 |
| Control | 2.3 | 9.38 | 2.10 | 0.68 | 4.32 | 0.31 |
| LSD (P = 0.05) | 0.6 | 1.67 | 0.39 | 0.55 | 1.37 | 0.18 |

[1]Biological treatments were incorporated into Pro mix (soil-less) at 1:40 (v/v) and placed into Styrofoam transplant flats and then seeded with tomato cv. Solar Set. There were four replicated flats per treatment.
[2]Seedling vigor was rated at 3 weeks after seeding on a scale of 1-5; 1 = poor, 2 = average, 3 = good, 4 = very good and 5 = excellent. Mean of 5 replications.
[3]Seedling height from the soil level to the tip. Mean of 4 replications, 3 seedlings per replication.
[4]Stem diameter is the mean of 4 replications, 6 seedlings per replication.
[5, 7] Seedling shoot and root fresh weight. Mean of 4 replications, 6 seedlings per replication.
[6]Number of leaflets per plant. Mean of 4 replications, 6 leaflets per replication. Largest leaflet surface area (usually from the 4th or 5$^{th}$ true leaf). Mean of 4 replications, 6 leaflets per replication.
Means followed by different letters are significantly different according to the protected least significance difference (LSD) test at P = 0.05.

**Table 2**. Effect of LS213 in different soil types 5 weeks after seeding

| Treatments[1] | Height (cm)[2] | Number of leaflets[3] | Stem diameter (mm)[4] | Shoot fresh weight (g)[5] | Leaflet surface area ($cm^2$)[6] |
|---|---|---|---|---|---|
| F - Chitosan | 2.25 b | 0.67 b | 0.40 ab | 0.013 b | 0.13 b |
| - LS213 | 3.30 a | 1.50 a | 0.59 a | 0.039 a | 0.41 a |
| - Control | 2.25 b | 0.75 b | 0.30 b | 0.025 ab | 0.16 b |
| LSD (P=.05) | 0.53 | 0.63 | 0.13 | 0.013 | 0.30 |
| | | | | | |
| P - Chitosan | 18.60 b | 26.09 a | 3.90 a | 3.97 a | 6.88 a |
| - LS213 | 21.59 a | 25.81 a | 4.07 a | 5.20 a | 8.18 a |
| - Control | 10.17 c | 7.67 b | 2.16 b | 0.59 b | 3.30 b |
| LSD (P=.05) | 2.67 | 3.4 | 0.43 | 0.96 | 1.76 |
| | | | | | |
| V - Chitosan | 8.75 a | 9.23 b | 1.76 ab | 0.40 b | 2.05 a |
| - LS213 | 9.38 a | 11.75 a | 1.90 a | 0.54 a | 2.19 a |
| - Control | 6.35 b | 4.8 c | 1.50 b | 0.17 c | 1.28 b |
| LSD (P=.05) | 1.10 | 1.23 | 0.35 | 0.19 | 0.52 |
| | | | | | |
| S - Chitosan | 13.00 b | 19.08 b | 2.90 b | 1.83 a | 3.69 a |
| - LS213 | 16.45 a | 23.42 a | 3.41 a | 3.06 a | 4.62 a |
| - Control | 5.70 c | 5.30 c | 1.22 c | 0.14 b | 1.11 b |
| LSD (P=.05) | 2.00 | 2.5 | 0.44 | 0.73 | 1.00 |

[1]Biological treatments were incorporated into four different soil-less mixes ( F = Fondmix, P = Promix, V = Vermiculite, S = Speedling-mix) at 1:40 (v/v) LS213 and 2.5% chitin for and placed into Styrofoam transplant flats and then seeded with tomato cv. Solar Set. There were four replicated flats per treatment
[2]Seedling height from the soil level to the tip. Mean of 4 replications, 3 seedlings per replication.
[3]Number of leaflets per plant. Mean of 4 replications, 6 leaflets per replication.
[4]Stem diameter is the mean of 4 replications, 6 seedlings per replication.
[5]Seedling shoot fresh weight. Mean of 4 replications, 6 seedlings per replication.
[6]Largest leaflet surface area (usually from the $4^{th}$ or $5^{th}$ true leaf). Mean of 4 replications, 6 leaflets per replication.
Means followed by different letters are significantly different according to the protected least significance difference (LSD) test at P = 0.05.

**Table 3**. Response of tomato plants treated with LS213, chitosan or not subjected to any treatment to inoculation with CMV

| Visual Symptoms | % of infected plant | | | | | |
|---|---|---|---|---|---|---|
| | Control | | Chitin | | LS213 | |
| | 2 wks [1] | 3 wks [1] | 2 wks | 3 wks | 2 wks | 3 wks |
| Vein-clearing | 8 | 3 | 5 | 0 | 5 | 0 |
| Mosaic | 28 | 33 | 13 | 40 | 28 | 53 |
| Strap-leaf | 0 | 33 | 0 | 40 | 8 | 53 |
| Stunting | 0 | 20 | 0 | 5 | 0 | 0 |
| No Symptom | 70 | 65 | 85 | 35 | 73 | 48 |
| No. of flowers/plant | 0 | 0 | 0 | 28 | 0 | 80 |

Biological treatments were incorporated into Pro-mix at 1:40 (v/v) for LS213 and 2.5% for Chitin and placed into Styrofoam transplant flats and then seeded with tomato cv. Solar Set. There were four replicated flats per treatment Tomato was planted at Nov. 5, transplanted at Dec. 8, inoculated at Dec. 10 and measured at Dec. 22 and Jan. 8.
[1]weeks after CMV inoculation. Numbers are percent of plants with typical CMV symptoms.

**Table 4**. Growth of tomato plants treated with LS213, chitosan or not subjected to any treatment at 4 weeks post-inoculation with CMV.

| Treatments[1] | Height (cm)[2] | | Stem diameter (mm)[3] | | Shoot fresh weight (g)[4] | | Root fresh weight (g)[5] | |
|---|---|---|---|---|---|---|---|---|
| | CMV[6] | No-CMV[6] | CMV | No-CMV | CMV | No-CMV | CMV | No-CMV |
| Control | 25.45 a | 28.80 a | 4.87 a | 5.86 a | 13.84 a | 19.54 a | 2.34 a | 4.38 a |
| Chitin | 34.10 b | 41.05 b | 6.18 b | 6.63 b | 22.24 b | 26.35 b | 4.37 b | 6.57 b |
| LS213 | 36.75 b | 43.35 b | 5.89 b | 7.67 c | 25.11 c | 28.03 b | 5.61 c | 8.37 c |
| LSD | 3.39 | 3.01 | 0.47 | 0.76 | 2.3 | 3.49 | 0.75 | 4.50 |

[1]Biological treatments were incorporated into Pro mix at 1:40 (v/v) and placed into Styrofoam transplant flats and then seeded with tomato cv. Solar Set. There were four replicated flats per treatment.
Tomato was planted at Nov. 05, transplanted at Dec. 8, inoculated at Dec. 10 and measured at Dec. 22 and Jan. 8.
[2]Seedling height from the soil level to the tip. Mean of 4 replications, 3 seedlings per replication.
[3]Stem diameter is the mean of 4 replications, 6 seedlings per replication.
[4]Seedling shoot fresh weight. Mean of 4 replications, 6 seedlings per replication.
[5]Seedling root fresh weight. Mean of 4 replications, 6 seedlings per replication.
[6] **CMV-challenged tomato plants were divided into two groups. CMV; typical CMV symptom developed plant and No-CMV; No-symptom developed plant even after challenged with CMV.**
Means followed by different letters are significantly different according to the protected least significance difference (LSD) test at $P = 0.05$.

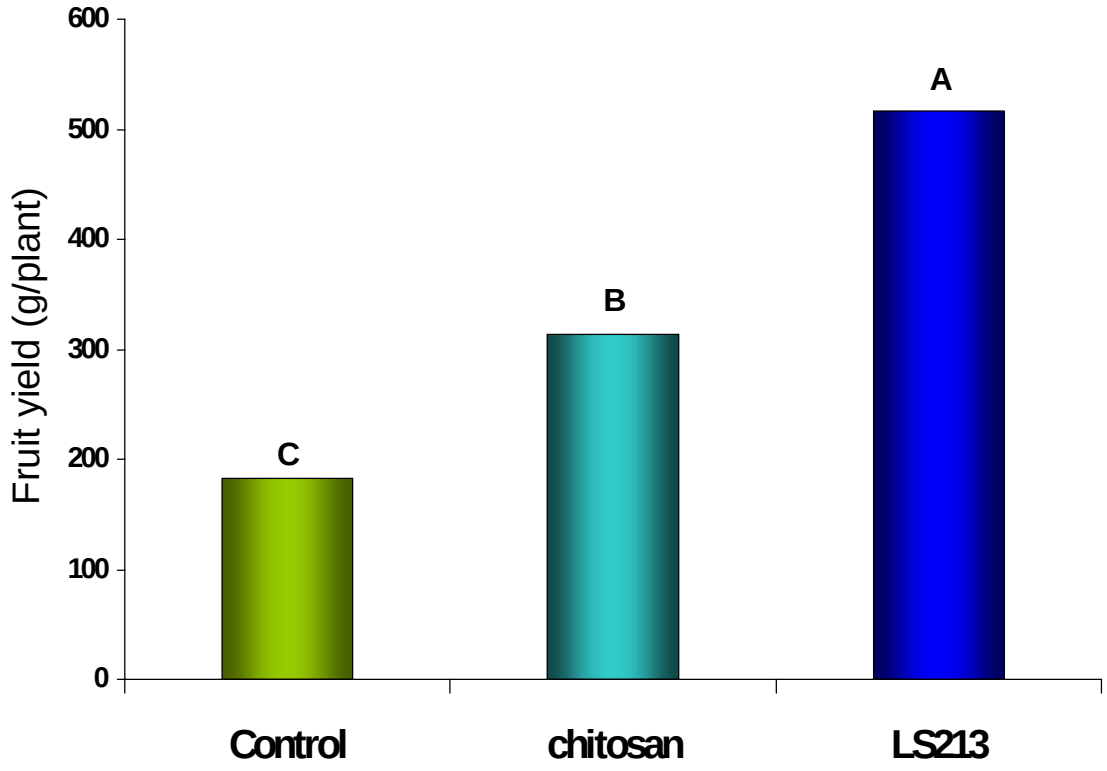


**Fig. 1**. Yield response of greenhouse-grown, CMV-infected tomato plants treated with LS213, chitosan or subjected to no treatment (control).

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