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In situ Evaluation of *Paenibacillus alvei* in Reducing Carriage of *Salmonella* Newport on Whole Tomato Plants

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ABSTRACT

Recently, tomatoes have been implicated as a primary vehicle in foodborne outbreaks of *Salmonella* Newport and other *Salmonella* serovars. Long-term intervention measures to reduce *Salmonella* prevalence on tomatoes remain elusive for growing and post-harvest environments. A naturally-occurring bacterium identified by 16S rDNA sequencing as *Paenibacillus alvei* was isolated epiphytically from plants native to the Virginia Eastern Shore tomato growing region. After initial antimicrobial activity screening against *Salmonella* and 10 other bacterial pathogens associated with the human food supply, strain TS-15 was further used to challenge an attenuated strain of *S.* Newport on inoculated fruits, leaves, and blossoms of tomato plants in an insect-screened high tunnel with a split-plot design. Survival of *Salmonella* after inoculation was measured for groups with and without the antagonist at days 0, 1, 2, 3, and 5 for blossoms and 6 for fruits and leaves, respectively. Strain TS-15 exhibited broad range antimicrobial activity against both major foodborne pathogens and major bacterial phytopathogens of tomato. After *P. alvei* strain TS-15 was applied onto the fruits, leaves, and blossoms of tomato plants, the concentration of *S.* Newport declined significantly ($p \leq 0.05$) compared with controls. Astonishingly, more than 90% of the plants had no detectable levels of *Salmonella* by day 5 for blossoms. The naturally-occurring antagonist strain TS-15 is highly effective in reducing carriage of *Salmonella* Newport on whole tomato plants. The application of *P. alvei* strain TS-15 is a promising approach for reducing the risk of *Salmonella* contamination during tomato production.

INTRODUCTION

The United States is one of the world's leading producers of tomatoes. Fresh and processed tomatoes account for more than $2 billion in annual farm cash receipts (http://www.ers.usda.gov/topics/crops/vegetables-pulses/tomatoes.aspx). U.S. fresh field-grown tomato production has consistently increased over the past several decades. Concurrently, an increasing number of outbreaks caused by various serovars of *Salmonella enterica* have been associated with consumption of fresh and fresh-cut tomatoes (1).

Contamination of produce can occur during field production or in the postharvest processing facility. Once contamination occurs, *S. enterica* serovars are able to survive on and in the tomato fruit despite the tomato's acidic interior (2-4). While a wide range of chemical sanitizers and physical treatments have been investigated with varying degrees of success for killing *Salmonella* on tomatoes at postharvest (5-7), no "kill-step" exists currently in processing that would eliminate *Salmonella* from contaminated tomatoes. At preharvest, there are no cultivars with resistance to other important diseases caused by plant pathogens that are also resistant to colonization of foodborne pathogens such as *Salmonella* (8). Following good agricultural practices (GAPs) (9) is the only available control right now to reduce the risk of tomatoes becoming contaminated with *Salmonella* in the field, indicating that additional interventions, such as biological control, are needed.

Biological control of plant diseases using microorganisms or their metabolites (10-12) offers a safe and effective alternative to the use of synthetic agrichemicals. The aim of this study was to isolate potential bacterial antagonists against *Salmonella*, to examine

70 their modes of action, and to test their effectiveness in reducing carriage of *Salmonella* on
71 whole tomato plants in a high tunnel setting.
72
73 **MATERIALS AND METHODS**
74 **Isolation and screening of antagonistic bacteria.** The native microflora of various
75 plant organs (including leaves, shoots, roots, and blossoms) and soil from various Eastern
76 Shore tomato growing locations were examined. Simply, three grams of plant material or
77 soil was mixed for 5 min in 1ml of phosphate-buffered saline (PBS). An aliquot (100 µl)
78 was plated onto Nutrient Yeast Glucose agar (NYGA). Ten colonies with unique
79 morphologies that developed within 48 h at 30°C under aerobic conditions were picked
80 for further purification and a 3% KOH test, to differentiate the Gram status without
81 staining (13). The pure cultures were then tested for antagonistic activity *in vitro* using an
82 agar plug method (14). Briefly, pour plates of each test organism were prepared by
83 mixing a 4 ml suspension of an overnight plate culture with sterile water in ca. 20 ml of
84 warm Tryptic Soy agar (TSA). After overnight incubation at 35°C, agar plugs were
85 punched from the agar with a sterile 10-mm stainless steel borer. Plugs were placed on
86 TSA agar containing a lawn of 10^6 cells of *Salmonella* Newport (15) and incubated at
87 35°C. Clear zones surrounding the plugs were measured at incubation periods of 24, 48,
88 and 96 h, respectively.
89 **Bacterial cultures.** Isolates of potential bacterial antagonists and indicator strains
90 (Table 1) were propagated on TSA at 35°C. Stock cultures grown overnight at 35°C on
91 TSA, were then resuspended in brain heart infusion broth (BHI) with 25% glycerol and
92 stored at -80°C. Three tomato plant associated bacterial pathogens including *Erwinia*

93 *carotovora* subsp. *carotovora, Pseudomonas syringae* pv. tomato strain dc3000, and
94 *Ralstonia solanacearum* race 5 were grown on TSA under 25°C (Table 1).
95 **Phenotypic and biochemical characterizations of potential bacterial antagonists.**
96 The morphological characteristics of potential bacterial antagonists were observed by
97 Gram and spore stains. These isolates were further tested with the Vitek® 2 compact
98 Biochemical Identification System (BioMerieux, Inc., Durham, NC) and the Biolog
99 Omnilog Microbial Identification System (Biolog, Hayward, CA) with GEN III
100 MicroPlates for biochemical properties according to manufactures' instructions.
101 **16S rRNA gene amplification, and sequencing.** Genomic DNA of potential bacterial
102 antagonists was extracted using the Wizard® genomic DNA purification kit (Promega,
103 Madison, WI). A pair of universal primers specific for bacterial 16S rRNA, Eubac27 and
104 R1492 (16), were used to amplify the corresponding gene. PCR amplification of the 16S
105 rRNA was performed with a Hotstart *Taq* plus DNA polymerase kit (QIAGEN, Valencia,
106 CA) under the following conditions: after an initial 5-min incubation at 95°C, the mixture
107 was subjected to 30 cycles, each including 1 min at 95°C, 1 min at 58°C, and 1 min at
108 72°C. A final extension was performed at 72°C for 10 min. Primers 4F, 27F, 357F, 578F,
109 1000R, and 1492R were used for sequencing (16) . The BLAST algorithm was used for a
110 homology search against Genbank. Only results from the highest-score queries were
111 considered for phylotype identification, with 99% minimum similarity (17).
112 **Determination of mode of action and spectrum of antimicrobial activities.** To
113 determine mode of action and antimicrobial spectrum of the bacterial antagonists, both
114 agar plug assay (using bacterial culture) and bioscreen assay (using culture supernatant)
115 were performed against a broad spectrum of major foodborne pathogens and bacterial

phytopathogens (Table 1). In the agar plug assay, bactericidal effects against pathogenic bacterial strains in the zone of inhibition were confirmed when no viable cells were recovered on TSA plates. In the bioscreen assay, the antagonist supernatant from overnight culture was filter sterilized with a 0.22 µm pore-size cellulose acetate (CA) membrane filter. Each 3 ml TS-15 cell-free culture supernatant (CFCS) was inoculated with 3 µl of 10^8 cfu/mL bacterial culture (Table 1). Aliquots (200 µl) were then dispensed into sterile Bioscreen C microwell plates (Growth Curves USA, Piscataway, NJ) and incubated as described for the respective bacterial strains. Bacterial growth was determined in five replicates by measuring $O.D._{600}$ at 20-min intervals for 24 hrs.

Tomato fruit assay. Red round ripe tomato fruits (130 ± 20 g each) were purchased from a local supermarket and refrigerated for no more than 3 days. Tomatoes were equilibrated to room temperature (RT) before testing and washed with 75% ethanol for surface sterilization and to remove any waxy residue if present. After air drying in a laminar flow hood, tomatoes were aseptically placed onto sterile metal trays with the stem scars facing down. A twenty microliter drop (18) of *S.* Newport overnight culture suspension (washed twice with PBS, and resuspended in 5 ml of PBS) was placed within a 3-cm-diameter circle on the side of the tomato, equidistant from both ends of the tomato. The *Salmonella* inoculum was allowed to dry before antagonist inoculation. A 40 µl drop of antagonist culture suspension (washed twice with PBS, and resuspended in 5 ml of fresh TSB) or 40 µl of TSB only was then placed on top of the *Salmonella* inoculum. After 1.5 h in the hood, completely air-dried samples were placed in a humidity chamber (*i.e.* a closed container filled with 1.5 L of water in a 30°C incubator). After 24 h incubation, each tomato was placed in a sterile Whirl-Pak™ filter bag

containing 30 ml of PBS and hand rubbed for 5 min to dislodge surface inoculated *Salmonella*. The wash suspension was diluted 10-fold in PBS and 0.1 ml aliquots of the appropriate dilutions were spread onto XLD agar (Becton Dickinson and Company, Sparks, MD) to determine the surviving *Salmonella* populations.

Field trials in high tunnel. (i) Plants. Trials were performed in 2010 (July through September) on tomato cultivar BHN602 in an insect-screened high tunnel at the United States Department of Agriculture (USDA), Beltsville Agricultural Research Center (BARC) north farm, Beltsville, Maryland. Tomato plants were grown from seeds in one of the BARC greenhouses in commercial organic peat mix (Johnny's 512 mix, Johnny's Selected Seeds, Fairfield, ME) and fertilized with Neptune's Harvest Organic Fish/Seaweed Blend fertilizer (Gloucester, MA) before and after transplanting. In the high tunnel, fertilizer was supplied from a single injector through drip tape supplemented with an OMRI-approved calcium source to prevent blossom end rot. Black plastic mulch was used to cover the 8 planting beds (2' x 20' each) over the drip tape. Planting slits were made in the black plastic at 15" intervals to accommodate 13 transplants per bed. Plants were staked using the Florida weave method with nylon support strings when 10" high. All plants were irrigated immediately after transplanting and at least weekly to achieve 1-1.5" water and meet fertility requirements. Soil moisture was monitored by irrometers and digitally on HOBO weather station that was located in the center of the high tunnel. Temperature, RH (Relative Humidity), PAR (Photosynthetically Active Radiation), and total SR (Solar Radiation) were monitored and recorded as well during this time.

(ii) Experimental design. A split-plot design was used with two treatments (*Salmonella* only and *Salmonella* with antagonist) as the first level sub-plot. Inoculation

sites including leaf, blossom, and tomato fruit were each assigned a second level sub-plot, with each inoculation site as an independent experimental unit; and day of harvest post-inoculation as a repeated measure. The second level corresponds to harvests used for 0 (2 hrs after inoculation as a benchmark for % recovery), 1, 2, 3, and 5 or 6 days persistence trials. Thirteen plants were planted in each plot. One plant on each end of each bed served as an uninoculated border plant, leaving 11 replicates per plot.

(iii) Inoculum preparation. Because of concerns about the safe use of pathogens in the field, an attenuated *S*. Newport strain #17 Δ*tolC*::*aph* was constructed for the high tunnel study. The *tolC* gene on the *S.* Newport strain #17 chromosome was replaced by a cassette containing a kanamycin resistance gene using the one-step inactivation method described by Datsenko and Wanner (19). TolC is an outer membrane protein not only important for the efflux of small compounds, but also for the export of large proteins. Disruption of *tolC* abolished the ability of *S*. Typhimurium to adhere, invade, and survive in eukaryotic cells (20). *S*. Enteritidis *tolC* mutant was shown to be avirulent in the BALB/c mouse model as well (21). TSB suspensions of *S*. Newport strain #17 Δ*tolC*::*aph* overnight culture were washed twice in PBS and then spot inoculated to three marked leaves (20 µl each), six to nine blossoms (10 µl each), and three breaker to red tomato fruits (20 µl each) for a final concentration of ~10^9 CFU/ml per plant. The inoculation spots were allowed to air dry (~ 1 h) before applying the antagonist. Antagonist cell suspensions were made from a bacterial lawn. After twice washing with PBS, cells were resuspended in 10 ml of TSB. Forty microliters of antagonist cell suspension or sterile TSB were applied to the same inoculation spot on leaves and fruits, and 10 µl to *Salmonella*-inoculated blossoms, of each plant in the 'with' or 'without'

antagonist group, respectively. Leaves, blossoms, and fruits were harvested on days 0, 1, 2, 3, and 5 (for blossoms) or 6 (for leaves and fruits) post inoculation (dpi).

(iv) Sample collection. Inoculated leaves, blossoms, and fruits from each plant were removed with sterile scissors and placed in individual plastic zipper bags, which were sealed and transported in an insulated cooler to the laboratory for analysis within 1 h. For leaves and blossoms, each sample bag was filled with 15 ml and 10 ml of PBS, respectively, and hand-rubbed for 3 min to dislodge surface populations of *Salmonella*. For fruits, each sample bag was filled with 30 ml of PBS and subjected to sonication at 55 Hz/min for 30 sec (22), which has been shown to be harmless to the infecting microorganisms. The PBS was diluted or concentrated through filtration (at later time points in the experiment) and surface plated (0.1 ml in duplicate) on TSA-*kan* (50µg/ml). Plates were incubated at 35°C overnight and counted for kanamycin-resistant colonies. Two colonies were randomly picked from each TSA-*kan* plate and confirmed by PCR using a set of verification primers (19).

(v) Statistical analysis. Estimates of the rate of reduction in bacterial counts were obtained by fitting a robust linear model of the log transformed CFU onto days (days after inoculation). The slopes of the fitted lines from antagonist treated and untreated surfaces were compared for differences in the rates of reduction. The analysis was performed using the R statistical software package, version 2.11.1, with the robust library. The results were tallied for each combination of plant location, antagonist, plant, and day. Within each plant location, both a regression and a rank test compared the effect of using the antagonist with that of not using it. Sum of the counts on all plates

207 divided by the sum of the volumes (0.1 ml) of the initial sample. An imputation
208 procedure, discussed by Blodgett (23), accounted for the TNTC plates.
209
210 **RESULTS**
211 **Isolation and identification of antagonistic bacteria.** A large number of
212 environmental isolates from the tomato field were screened for antimicrobial activity
213 against *S.* Newport. Two isolates, one from an epiphytic leaf surface of native Eastern
214 Shore vegetation and the other from Eastern Shore tomato soil, showed distinct inhibition
215 areas on basal TSA agar. These isolates formed pale colonies and swarmed vigorously on
216 TSA. Morphologically, the isolates were rod-shaped, 0.7-0.95 µm by 3.18-3.42 µm,
217 gram-positive bacteria. Upon prolonged incubation on an agar medium, cells produced
218 central endospores.
219 The isolates were positive for oxidase, nitrate reduction, gelatin liquefaction, starch
220 hydrolyzation, casein hydrolysis, glucose fermentation, and urease but negative for
221 catalase, indole production, and H_2S formation. The bacterium grew well in TSB broth
222 under aerobic conditions. Genomic analysis showed the 16S rRNA gene of both isolates
223 shares over 99.0% sequence similarity with that of *Paenibacillus alvei*. Biolog Gen III
224 MicroPlate confirmed the high similarity of both isolates (> 99%) with *P. alvei*. Thus, it
225 was concluded that both isolates belong to *P. alvei*, and they were given strain
226 designations of A6-6i and TS-15 respectively.
227 **Broad antimicrobial spectrum of *P. alvei* strains A6-6i and TS-15.** *In vitro* agar
228 plug assays showed inhibition zones against all the indicator strains including six major
229 foodborne pathogens and three major tomato bacterial phytopathogens when challenged

with both *P. alvei* isolates (Fig. 1A and 1B). Notably, the antagonist migrated outward from the plug after forming the inhibition zone with SD (*S. dysenteriae*) or LM (*L. monocytogenes*), and the antagonistic growth ring expanded with time, especially in the case of *Listeria*. Both A6-6i and TS-15 had a wide range of inhibition against MRSA strains with zone diameters from 15 to 35 mm, and 15 to 20 mm, respectively. It is also interesting to note that strain A6-6i showed strong inhibitory effects on various MRSA strains tested despite the fact that some strains were resistant to up to 14 different antimicrobial drugs.

When supernatants were tested against the panel of gram-negative and gram-positive bacteria using the Bioscreen assay, both A6-6i (Fig. 2) and TS-15 (not shown) CFCS exhibited a broad spectrum of antimicrobial activity, in which the lag phase was significantly extended in all the pathogens tested and the cell density was largely reduced at the end of incubation. Furthermore, the lag phase in CS (*C. sakazakii*), SD (*S. dysenteriae*), LM (*L. monocytogenes*), and some MRSA strains were extended to almost 24 h in both A6-6i and TS-15 CFCS. Compared to A6-6i, CFCS from TS-15 had much stronger inhibitory effect when tested against SN (*S.* Newport) (not shown).

Efficacy of *P. alvei* A6-6i and TS-15 on tomato fruit in humidity chambers. *S.* Newport showed significant reduction on the tomato fruit surface by both *P. alvei* strains A6-6i and TS-15. However, comparing an average of 0.5 log reduction by A6-6i, TS-15 had a 5 log reduction in the *S.* Newport population applied to tomato fruits (Fig 3). Numbers of *S.* Newport recovered from tomato surfaces were 100 times less on average when the antagonist was added prior to *Salmonella* on the tomato surface. Nevertheless, no significant difference was found in the rate of population decline regardless of

253 whether the antagonist was inoculated before (Fig. 3A) or after *S*. Newport inoculation
254 (Fig. 3B), pointing to a potential bactericidal mode of action rather than simple
255 competitive exclusion.

256 **Field trials in high tunnel using *P. alvei* TS-15.** Based on the results from tomato
257 fruit assay, *P. alvei* strain TS-15 was selected for further high tunnel field trials. During
258 field trials from July through September 2010, the maximum daily temperature and RH
259 varied, respectively, between 26.7 and 37.8°C and between 56% and 80% (available at
260 www.wunderground.com/history/). At Day 0, variations were detected between the group
261 without TS-15 and the group with TS-15 on leaf and blossom but not on tomato in terms
262 of *Salmonella* population after inoculation (Fig. 4). Taking all the variations into effect,
263 the concentration of *Salmonella* was significantly lower ($p \leq 0.05$) on plants with TS-15 on
264 leaves, blossoms, and fruits from 1 to 5 (for blossom) or 6 (for leaf and tomato) dpi (Fig.
265 4). Notably, close to 100% of the '*Salmonella* only' plants still had detectable levels of
266 *Salmonella* at the end of the blossom and leaf trials, whereas only 2 plants (< 20%) had
267 detectable levels of *Salmonella* in the 'antagonist group' in the blossom trial and 6 plants
268 (~ 50%) in the leaf trial. Moreover, the rate of decrease in bacterial concentration was
269 significantly greater ($p \leq 0.05$) on leaves and blossoms with TS-15 versus those without
270 TS-15; decreases were 12-fold per day versus 2.7-fold per day for leaves, and 8.9-fold
271 versus 1.4-fold for blossoms, respectively (Fig. 5). Nevertheless, no statistically
272 significant difference was found in the mortality rate of *Salmonella* on tomato fruits.
273

274 DISCUSSION

Contaminated tomatoes have been implicated in several high profile outbreaks in the U.S. (24, 25), and *Salmonella enterica* serovar Newport is amongst the most recurring serovars implicated in foodborne outbreaks associated with tomatoes (26, 27). Extensive research has been done to show that *Salmonella* can contaminate tomato fruit at the primary production level through soil, irrigation water, and blossoms (4, 28-30), allowing the pathogen to colonize the exterior and interior of developing fruit. Due to the risk of internalization, *Salmonella* needs to be controlled at the farm level. Biological control has been widely applied to suppress plant diseases caused by phytopathogens (31, 32). However, few have been reported to control human foodborne pathogens on produce especially at the preharvest level. With only 1- to 2-log reductions, limited success was achieved using bacteriophages as biocontrol agents (33-35). *Enterobacter asburiae* strain JX1demonstrated over a 5-log reduction in the growth of *Salmonella* in the rhizosphere of tomato plants and on the developing fruit (34), however, this bacterium can cause an array of diseases in humans itself (36), making it an undesirable candidate for commercial commodities destined for the human food supply. In this study, two new bacterial strains, A6-6i and TS-15, exhibiting substantial antimicrobial efficacy against a broad range of foodborne pathogens and tomato bacterial phytopathogens, were identified as *P. alvei*, a bacterium very rarely associated with human infections (37). Results of the *in situ* tomato plant trials further showed that *P. alvei* strain TS-15 is highly effective in reducing the carriage of *S.* Newport on tomato plants, indicating its potential use as a novel biocontrol agent to mitigate *Salmonella* contamination at the preharvest level.

297 The antagonist may exhibit competitive exclusion over certain foodborne pathogens;
298 many *Paenibacillus* species are already part of the natural microbial community in soil,
299 water, and rhizosphere of various plants (38). Results from the bioscreen and agar plug
300 assays, however, indicate that the inhibitory (*i.e.* bacteriostatic and bacteriocidal) effects
301 of this antagonist on foodborne pathogens can be mainly attributed to its antimicrobial
302 activities. Whole genome sequencing was performed to help identification of diverse
303 antibiotic biosynthetic genes present in these two isolates (39). A few novel antimicrobial
304 agents, with activities against many foodborne pathogens including *Salmonella spp.*, *E.*
305 *coli* O157:H7, *L. monocytogenes*, *S. dysenteriae*, *C. sakazakii*, and multi-drug resistant *S.*
306 *aureus*, were discovered in our laboratory (unpublished data).
307 In the high tunnel trial, *P. alvei* TS-15 was much more effective in suppressing the
308 growth of *Salmonella* on blossoms and on leaves than on tomato fruits. However, this
309 seemed more to do with the lack of persistence of *Salmonella* on tomato fruit surfaces
310 rather than any lack of *Paenibacillus* activity. The mortality rate in the *Salmonella*
311 control group was much higher on tomato fruits compared to that for leaves and
312 blossoms. In general, the smooth, waxy surfaces of developing tomato fruits was
313 associated with reduced survival of microbes (40-42), and this has implication for the
314 application strategy for this biocontrol agent. Despite this caveat in the tomato surface
315 study, the effectiveness of biological intervention has been shown to be greatly impacted
316 by the ratio of antagonist to pathogen in culture (43). Provided that the ratio of *P. alvei*
317 TS-15 to *S.* Newport was only 1:1 in the fruit assay and high tunnel trial and the actual
318 level of *Salmonella* on naturally contaminated produce is much lower than 6 log CFU/g
319 of tissue, application of *P. alvei* TS-15 at 6 log CFU/g of tissue as used in this study

should be more than sufficient to inhibit the growth of *Salmonella* on tomato to a clinically significant level.

In summary, the results of this study have demonstrated the efficacy of *P. alvei* TS-15 against *Salmonella* on the blossoms and leaves of tomato plants. In order to more successfully apply such an agent, studies will be conducted to determine the efficacy of *P. alvei* TS-15 in suppressing *Salmonella* in the rhizosphere of tomato plants, to develop formulation *P. alvei* TS-15 for use against *Salmonella* and other foodborne pathogens on produce crops, and to assess the biological safety due to environmental and human exposure to this organism incidentally in the food and feed supply. Such studies will ascertain suitability of this promising new microbial agent as an early intervention tool in our battle against *Salmonella* contamination of fresh-cut produce.

336 **Table 1.** Strains used in the present study
337

Strain	Reference or source
Salmonella enterica subsp. enterica serovar Newport #17	CFSAN laboratory collection
Salmonella enterica subsp. enterica Saintpaul	CFSAN laboratory collection
Salmonella enterica subsp. enterica Montevideo 42N	CFSAN laboratory collection
Salmonella enterica subsp. enterica Javiana	CFSAN laboratory collection
Salmonella enterica subsp. enterica Typhimurium 368477	CFSAN laboratory collection
Salmonella enterica subsp. enterica Typhimurium SAR C #1	SGSC[a]
Salmonella enterica subsp. enterica Typhi SAR C #3	SGSC
Salmonella enterica subsp. arizonae SAR C #5	SGSC
Salmonella enterica subsp. arizonae SAR C #7	SGSC
Salmonella enterica subsp. arizonae SAR C #9	SGSC
Salmonella bongori SAR C #11	SGSC
Salmonella bongori SAR C #13	SGSC
Salmonella bongori SAR C #15	SGSC
Escherichia coli O157:H7 IS O57	CFSAN laboratory collection
Escherichia coli O157:H7 EDL933	CFSAN laboratory collection
Escherichia coli ATCC 51434	ATCC[b]
Escherichia coli ATCC BAA-179	ATCC
Shigella dysenteriae 2457T	CFSAN laboratory collection
Shigella dysenteriae BS103	CFSAN laboratory collection
Cronobacter sakazakii E932	CFSAN laboratory collection
Cronobacter sakazakii E784	CFSAN laboratory collection
Listeria monocytogenes N1-225	CFSAN laboratory collection
Listeria monocytogenes R2-583	CFSAN laboratory collection
Methicillin-resistant *Staphylococcus aureus* #9	CFSAN laboratory collection
Methicillin-resistant *Staphylococcu aureus* #12	CFSAN laboratory collection
Methicillin-resistant *Staphylococcu aureus* #28	CFSAN laboratory collection
Methicillin-resistant *Staphylococcu aureus* #29	CFSAN laboratory collection
Methicillin-resistant *Staphylococcu aureus* #30	CFSAN laboratory collection
Staphylococcu aureus NRS70	NARSA[c]
Staphylococcu aureus NRS106	NARSA
Staphylococcu aureus NRS107	NARSA
Staphylococcu aureus NRS271	NARSA
Salmonella enterica Newport #17 Δ*tolC*::*aph*	CFSAN laboratory collection
Erwinia carotovora subsp. *carotovora*	Dr. Dilip Lakshman, ARS[d]
Pseudomonas syringae pv. tomato strain dc3000	Dr. Dilip Lakshman, ARS
Ralstonia solanacearum race 5	Dr. Dilip Lakshman, ARS

338 [a] SGSC, Salmonella Genetic Stock Centre, University of Calgary, Canada

[b] ATCC, American Type Culture Collection, Manassas, VA, USA
[c] NARSA, Network on Antimicrobial Resistance in *Staphylococcus aureus*, Chantilly, VA, USA
[d] ARS, Agricultural Research Service, Department of Agriculture, Beltsville, MD, USA

346 **Figure legends:**

347 **Figure 1. *In vitro* inhibition of foodborne pathogens and tomato bacterial**
348 **phytopathogens by *Paenibacillus alvei* A6-6i and TS-15 on tryptic soy agar (TSA).**
349 The inhibition zones (mm) were measured against strains from *Salmonella* spp.,
350 *Escherichia coli* (*E. coli*), *Cronobacter sakazakii* (CS), *Listeria monocytogenes* (LM),
351 *Shigella dysenteriae* (SD), Methicillin sensitive *Staphylococcus aureus* (MSSA),
352 Methicillin resistant *Staphylococcus aureus* (MRSA), *Ralstonia solanacearum* race 5 (*R.*
353 *solanacearum*), *Pseudomonas syringae* pv. tomato strain dc3000 (*P. syringae*), and
354 *Erwinia carotovora* subsp. *carotovora* (*E. carotovora*). The plot represents the lowest,
355 highest, and average measurements in each of the species listed above. The experiment
356 was repeated twice.
357
358 **Figure 2. Growth inhibition of major foodborne pathogens in *P. alvei* A6-6i cell free**
359 **culture supernatant (CFCS).** Brain Heart Infusion (BHI) broth was used as a control.
360 Bacterial growth of A) *L. monocytogenes* (LM), *S. dysenteriae* (SD), *E. coli* O157, *C.*
361 *sakazakii* (CS), and *S.* Newport strains; and B) Methicillin resistant *S. aureus* (MRSA)
362 strains in *P. alvei* A6-6i CFCS and BHI was determined in five replicates by measuring
363 $O.D._{600}$ at 20-min intervals for 24 hrs. The experiment was repeated twice.
364
365 **Figure 3. Recovery of *S.* Newport from intact tomato fruit surfaces after treatment**
366 **with antagonist inoculations over 24 h at 30°C in humidity chamber.** A) Recovery of
367 *S.* Newport with *S.* Newport inoculated first; B) Recovery of *S.* Newport with antagonist

368 inoculated first. Error bars represent the ±standard deviation of the means of two
369 experiments each with 10 replicates (n=20).
370
371 **Figure 4. Recovery of an *S.* Newport attenuated strain from tomato plants including**
372 **leaves, blossoms, and tomato fruits.** In the high tunnel study, *S.* Newport was recovered
373 from leaves, blossoms and tomato fruits 0, 1, 2, 3, and 5 (for blossoms) or 6 (for leaves
374 and tomatoe fruits) dpi with *S.* Newport only or *S.* Newport plus antagonist co-
375 inoculation. The results were tallied for each combination of plant location, antagonist,
376 plant, and day. Estimated recovery of *S.* Newport on each sample point from log
377 transformed data in control (-) and antagonist treatment (+) panel was scatter plotted for
378 leaf (A), tomato fruit (B), and blossom (C).
379
380 **Figure 5. The rate of decrease in *S.* Newport concentration post inoculation on**
381 **leaves, blossoms, and tomato fruits.** In high tunnel setting, leaves, blossoms and tomato
382 fruits were harvested on 0, 1, 2, 3, and 5 (for blossoms) or 6 (for leaves and tomato fruits)
383 dpi to recover any remaining *S.* Newport. The rate of decrease in *S.* Newport
384 concentration in control (light grey bar) and antagonist treatment (dark grey bar) groups
385 was calculated and compared. Results were shown as means with +/- 2SE. * represents
386 that the rate of decrease in *S.* Newport concentration was significantly greater ($P<0.05$)
387 compared with that in the control group.
388

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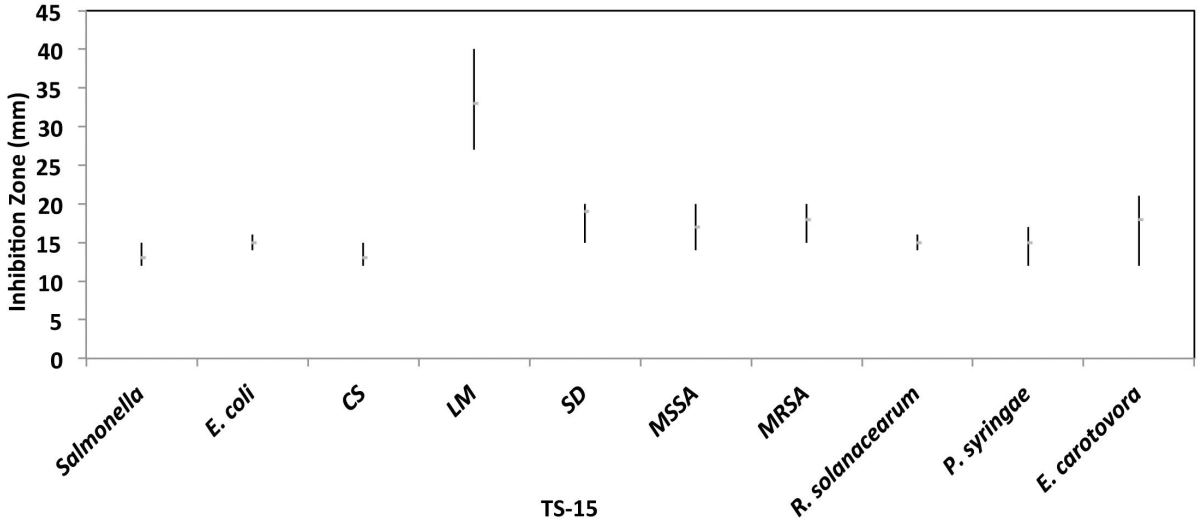
A)



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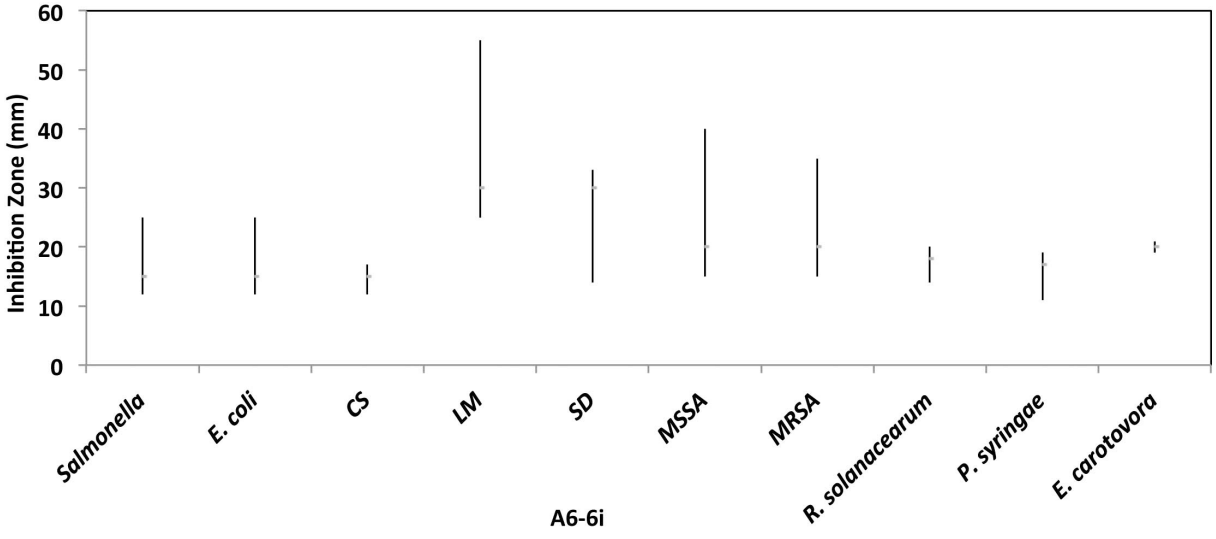

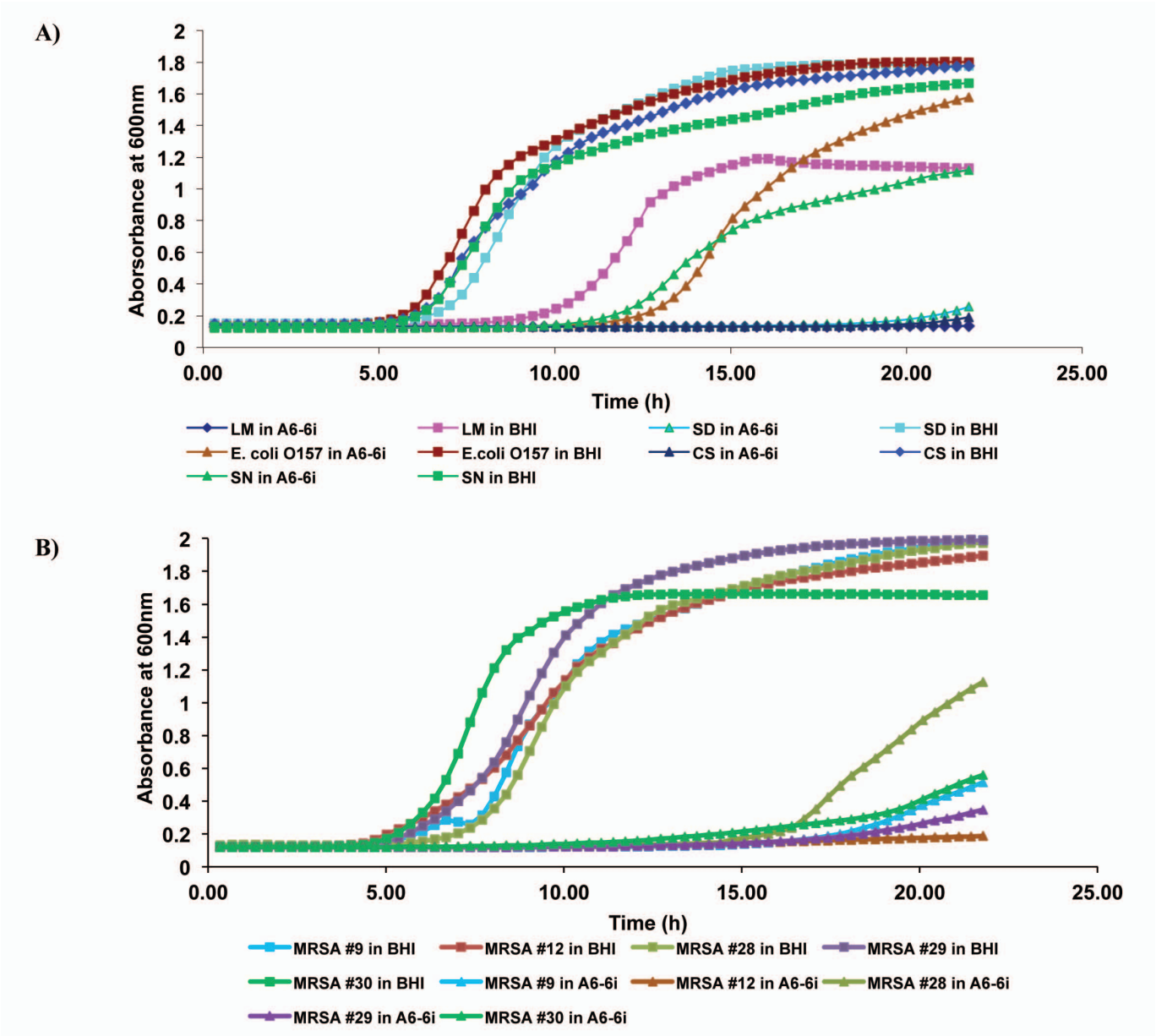

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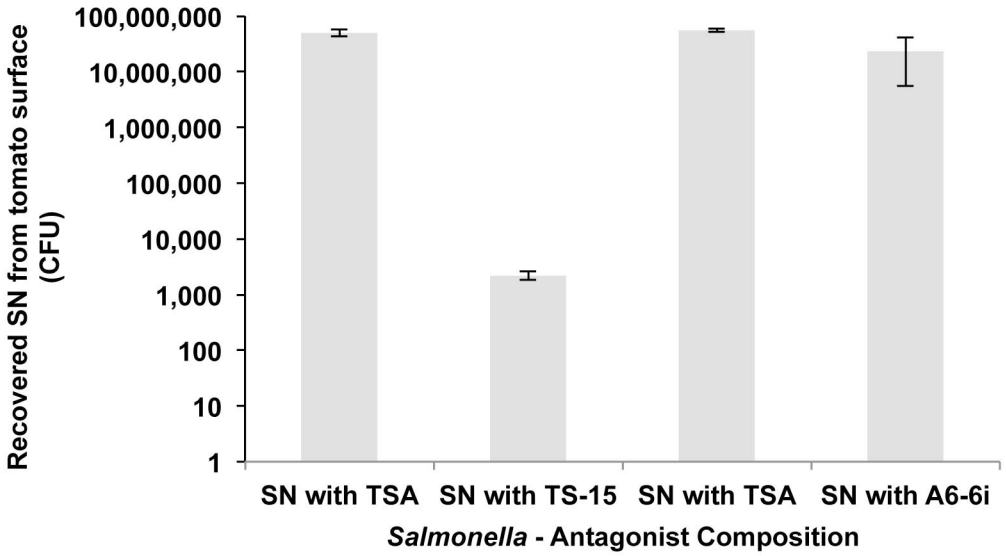

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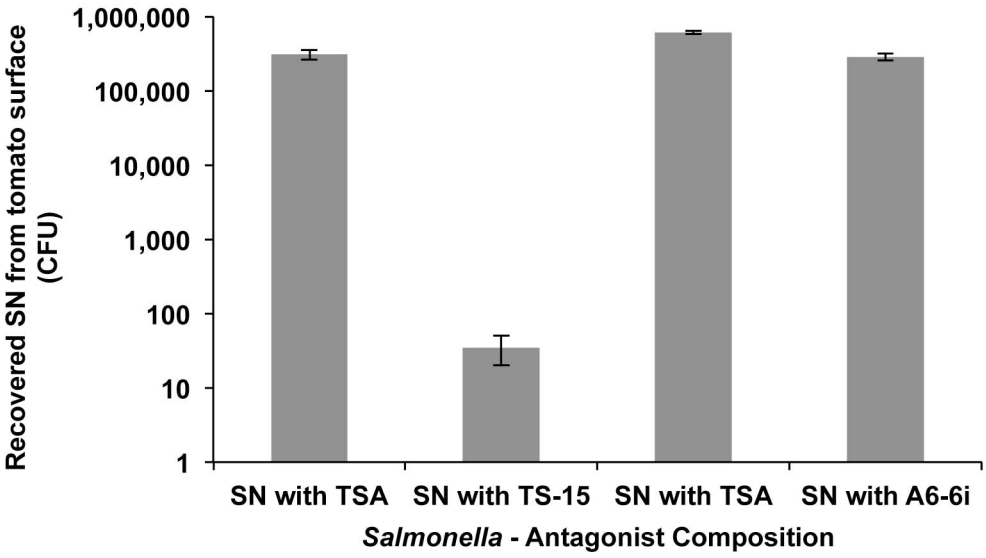

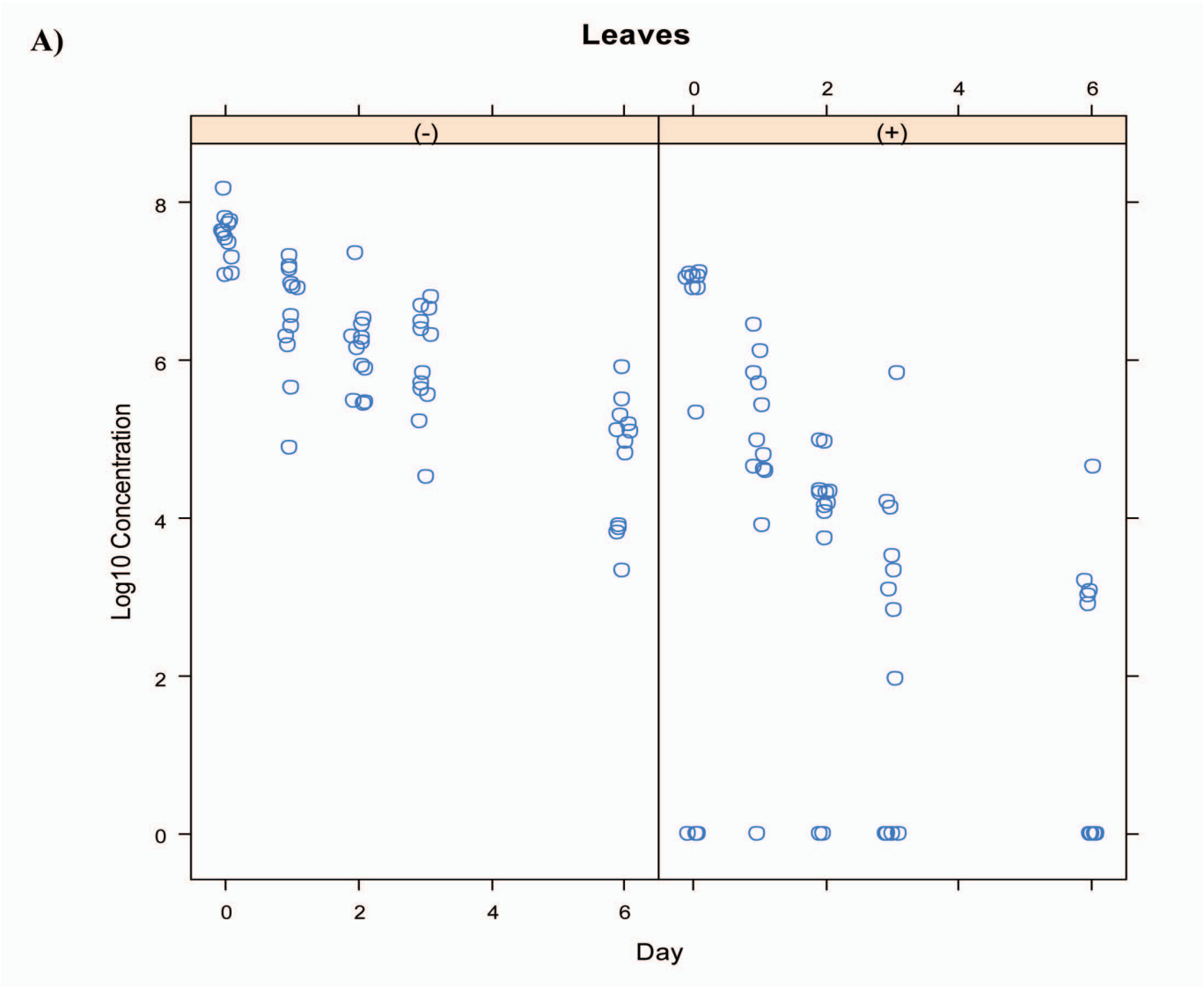

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