

Article

Effect of temperature on *Salmonella Javiana*, *Listeria monocytogenes*, and *Listeria innocua* persistence in hydroponic nutrient solution

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Abstract: This study aimed to determine the persistence of *Salmonella Javiana*, *Listeria monocytogenes*, and *Listeria innocua* in hydroponic nutrient solution (NS) at 15, 25, 30, and 37°C over a 21-day period to mimic time from seedling to mature lettuce. Bacteria were inoculated in modified Hoagland's NS at 10⁶ CFU/ml and maintained at 15, 25, 30, and 37°C. Samples were collected at various time points and quantified. Data were analyzed using a mixed effect model to compare mean log CFU/ml obtained from each sampling point for all three bacteria at four different temperatures. Least mean squares were calculated to compare mean log CFU/ml. Tukey-Kramer honest significant difference test was used to compare mean values. At all temperatures, *S. Javiana* persisted in NS throughout the 21-day study period, compared to *L. innocua* and *L. monocytogenes* where persistence was limited to d 5 to d 14 and d 1 to d 14, respectively. Similarly, decimal reduction values (D-values) of *S. Javiana* indicated longer persistence in nutrient solution than *L. innocua* and *L. monocytogenes* at most temperatures. For instance, at 15°C and 25°C D-values for *S. Javiana* were estimated at 82 and 26 d, respectively, compared to D-values of 3.6 and ~3 d for *L. monocytogenes*. Data indicate that temperature has minimal effect on *S. Javiana* and thus may pose a greater risk during hydroponic production of leafy greens due to longer survival in NS when compared to *Listeria* spp. This study furthers the understanding of potential food safety risks associated with hydroponic systems and may aid in developing management strategies to reduce foodborne outbreaks, fresh produce recalls, and economic losses.

Keywords: Enteric pathogens; hydroponic; nutrient solution; *Salmonella*; *Listeria monocytogenes*; *Listeria innocua*; lettuce; temperature

1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), by 2050 arable land per person is expected to diminish to one-third of the land that was available in 1970 [1]. Factors that may lead to depletion of arable lands include climatic changes, increase in urbanization, population growth, reduction in freshwater resources, soil fertility loss, and over-farming practices [1]. To meet the consumer's increasing demand for fresh produce year-round, growers are adopting controlled environment agriculture (CEA) production systems.

Controlled environment agriculture includes various structures (e.g., greenhouses and warehouses) and growing systems (e.g., hydroponic systems and soilless substrates) as an alternative cultivation method to traditional, in-field production. In CEA systems, environmental conditions such as temperature, light, humidity, and pH are typically maintained by automated control systems making it favorable for the growth of fresh produce.

Hydroponic production is a system where plants are grown by dipping their roots partially or fully into nutrient solution containing macro- and micronutrients. These nutrients are the same as those found natu-

rally in the soil which promote the development of plants in the field. There are different types of soil free systems such as wicking, deep water culture (DWC), drip, ebb and flow, nutrient film technique (NFT), and aeroponics. Some advantages and disadvantages of the listed hydroponic systems have been discussed by [2]. Although hydroponic cultivation takes place in closed and controlled environments, these systems still face microbial contamination risks including contaminated water used for irrigation, workers' personal hygiene, internal and external hygienic conditions of the growing space, and improper, or lack of, sanitization of hydroponic systems between the production cycles. Although foodborne disease outbreaks related to hydroponically grown fresh produce are limited [3], it is essential to develop and instill food safety strategies to mitigate microbial contamination risks for such systems [4].

A review by Riggio *et al.* (2019) indicated that distinct types of hydroponic systems have been used to study the internalization and dissemination of human enteric (bacterial and viral) pathogens during hydroponic cultivation. Since water is the essential component of hydroponic cultivation and is used in the preparation of nutrient solution, contamination of leafy greens via irrigation water containing human pathogens such as *Salmonella enterica* subsp. *enterica*, *Listeria monocytogenes*, and human norovirus (HuNoV) may lead to food safety risks during hydroponic production [5-8]. Studies have shown that the route of entry for human pathogens into fresh produce can be via plant roots that are immersed in hydroponic nutrient solution [9-13]. However, there are little to no studies [14-16] focusing on bacterial survival in hydroponic nutrient solution in the absence of plants within the system. Of the studies that have considered bacterial survival in nutrient solution, none have evaluated the impact of seasonal temperature variations. Therefore, the present study aimed to investigate the persistence of human bacterial pathogens in hydroponic nutrient solution subjected to different temperatures. During this study, experiments were performed over a 21-day period as a preliminary step for investigating the persistence and internalization of human pathogens in a recirculating DWC hydroponic systems used for the production of lettuce. This 21-day period corresponds to the timeframe for the development of lettuce from seedling to mature plant stage thus mimicking a more realistic scenario of hydroponic cultivation.

2. Materials and Methods

2.1 Preparation of bacterial cultures

Bacterial cultures used in this study include *Listeria monocytogenes* (FSL R2-574, strain F2365, Cornell University, Institute for the Advancement of Food and Nutrition Sciences (IAFNS) Collection), *Listeria innocua* (FSL C2-0008, Cornell University; IAFNS Collection), and *Salmonella enterica* subsp. *enterica* serotype Javiana (ATCC BAA1593, American Type Culture Collection, Manassas, VA). *Salmonella* Javiana from frozen 50% glycerol stock was streaked onto tryptic soy agar (TSA; Difco, Sparks, MD) plates using sterile inoculation loops followed by incubation for 18 to 24 h at 37°C. *Listeria monocytogenes* and *L. innocua* were also taken from frozen 50% glycerol stocks, streaked onto separate brain heart infusion (BHI; BD, Franklin Lakes, NJ) agar plates, and incubated for 24 h at 35°C. Pure isolated colonies from the streaked plates were cultured separately in 10 ml of BHI broth (*Listeria* spp.), and *S. Javiana* into tryptic soy broth (TSB; Difco) for 24 h at 35°C with shaking at 125 rpm. Bacterial cells for each bacteria species were harvested by centrifugation at 4000 x g for 10 min at 4°C and washed twice in 10 ml of 1X Phosphate Buffer Saline (PBS, pH 7.4) solution. The final cell pellet was re-suspended in 10 ml 1X PBS solution at approximately 9 log colony forming units (CFU)/ml. *L. innocua* was utilized in this study to validate whether it would serve as an appropriate surrogate for *L. monocytogenes* during our present and the future studies. This strain of *L. innocua* is also determined to be an appropriate surrogate for use in produce safety research based on the curated list of produce relevant strains published by Harrand *et al.* [17]

2.2. Preparation of nutrient solution

Five hundred milliliters of nutrient solution were prepared by mixing 7.5 ml of stock solution containing macronutrients and micronutrients (Table 1), 0.1 ml sodium thiosulphate (Sigma Chemical Co., St. Louis, MO) stock solution

(12.5 ppm), and 492.4 ml of tap water. The average pH and electrical conductivity of nutrient solution during this study was 6.01 ± 0.07 and 2.62 ± 0.09 mS/cm.

2.3. Inoculation of nutrient solution

Each of the three separate 250 ml glass bottles received 100 ml nutrient solution. One hundred microliters of each bacterium ($9 \log$ CFU/ml) were inoculated in separate bottles containing the prepared nutrient solution. The final bacterial concentration was approximately $6 \log$ CFU/ml. The three glass bottles with inoculated nutrient solution and a glass bottle containing 100 ml nutrient solution only (negative control) were placed in a shaking incubator. The incubator was maintained at 15, 25, 30, or 37°C with shaking at 50 rpm throughout 21 days of study. This 21-day study period represents the growth stage of lettuce from seedling to mature plant.

Table 1. Modified Hoagland's nutrient stock solution containing macronutrients and micronutrients. .

	Stock Solution	Fertilizer salts	Conc. (grams/L)
Macronutrients*	A	Calcium nitrate	208.75
		Potassium nitrate	65.00
	B	Mono potassium phosphate	28.49
		Magnesium sulfate	100.63
		Potassium chloride	27.50
Micronutrients†	C	Fe-EDTA	3.00
		Mn-EDTA	1.50
		Boric acid	0.50
		Copper sulfate	0.38
		Zinc sulfate	0.28
		Sodium ammonium molybdate	0.05

Fe-EDTA = Ferric-Ethylenediaminetetraacetic acid (EDTA), Mn-EDTA = Manganese EDTA .

*Macronutrients (Haifa Group, Altamonte Springs, FL) .

†Micronutrients (JR Peters, Inc, Allenstown, PA) .

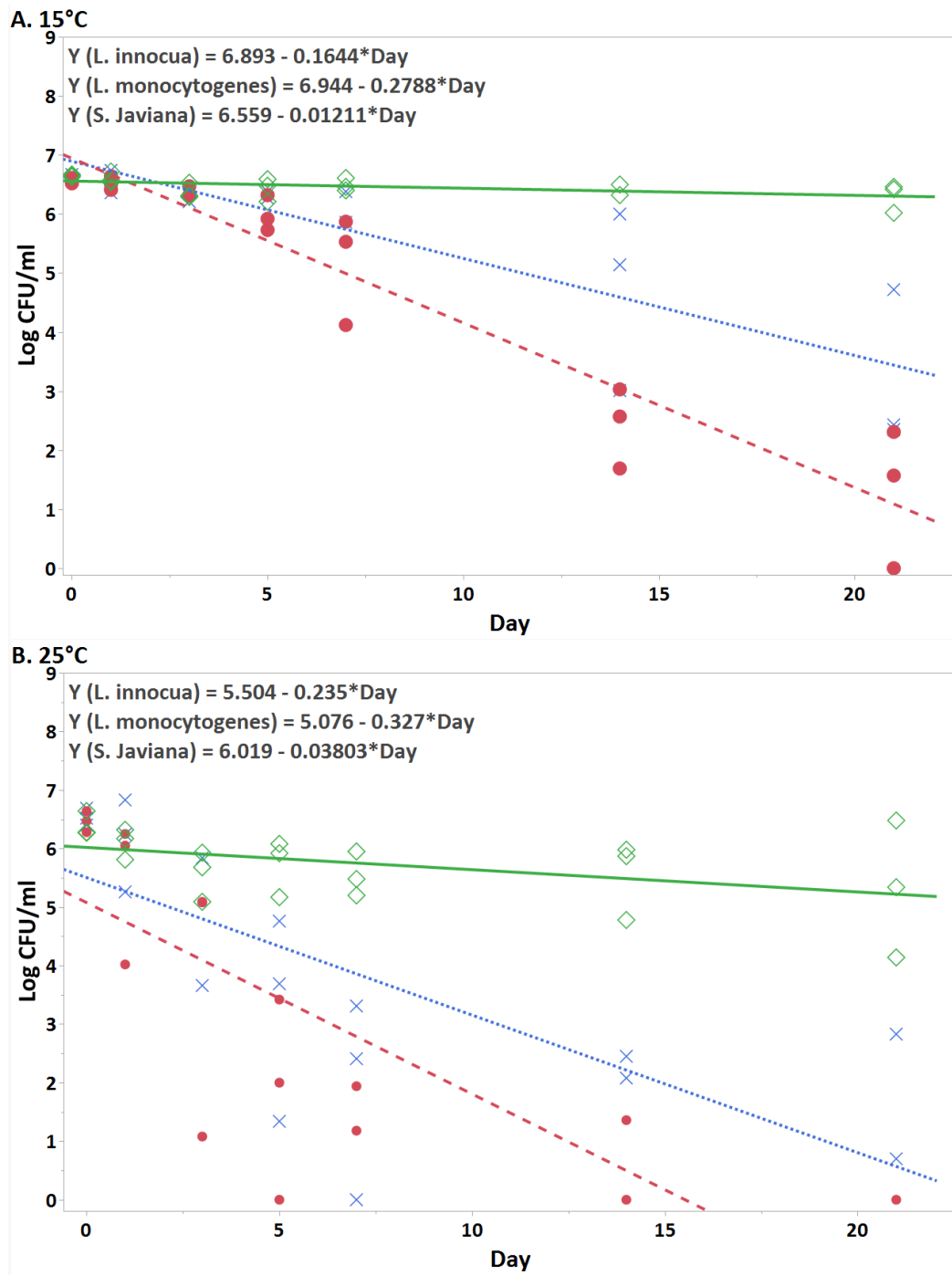
2.4. Sample collection and bacterial quantification

Post inoculation, 1 ml of sample was collected from all four glass bottles on day 0, 1, 3, 5, 7, 14, and 21 and serially diluted. One hundred microliters of each dilution were plated on Oxford Listeria Agar Base with modified Oxford Listeria supplement (MOX; Difco) for detection of *L. monocytogenes* and *L. innocua*, and Xylose Lysine Tergitol™ 4 agar (XLT4) plates (Hardy Diagnostics, Santa Maria, CA) were used for detection of *S. Javiana*. Colony forming units were counted and reported as log CFU/ml.

2.5. Data analysis

All data were analyzed using JMP® Pro 16 (SAS Institute, Inc., Cary, NC, USA) software. Three trials (n=3) were performed for each temperature (15, 25, 30, or 37°C). The persistence data of each bacterium in nutrient solution was log transformed prior to analysis. This study was a completely randomized block design with a split-split plot, and data were analyzed using a mixed effect model to compare mean log CFU/ml obtained from each sampling point for all three bacteria at four different temperatures. Least mean squares were calculated to compare mean log CFU/ml. Tukey-Kramer honest significant difference test was used to compare mean values. A value of $p < 0.05$ was considered as statistically significant. Each data point (log CFU/ml) of individual bacterium from each temperature was used for the scatter plot, and decimal reduction values (*D*-values) were calculated using the slope from the linear regression equa-

tion (Figure 1). Here, D -values indicate one log (90%) reduction of bacteria (in days) in hydroponic nutrient solution for each temperature.



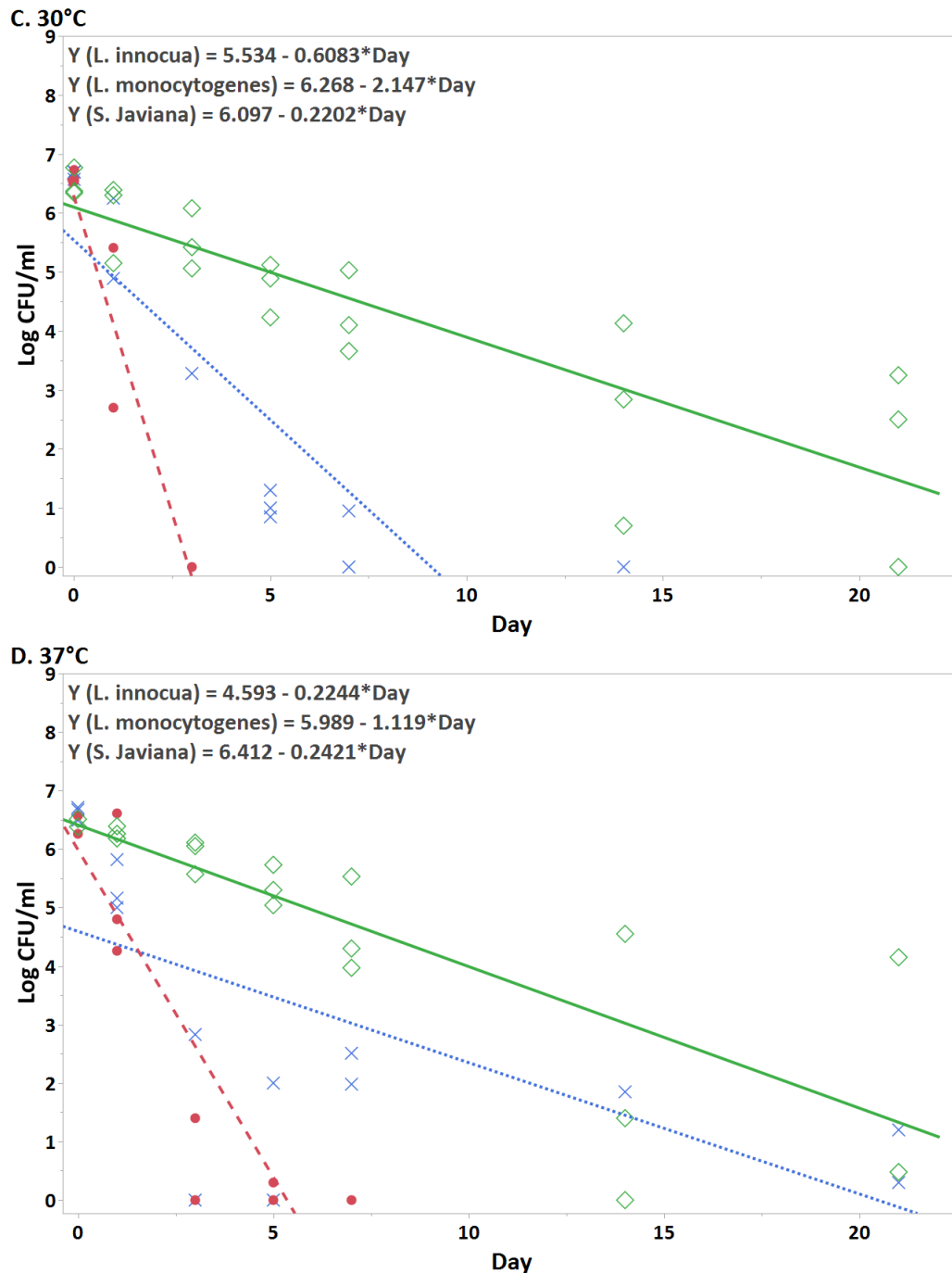


Figure 1. Persistence of *L. innocua* (blue 'x' and dotted line), *L. monocytogenes* (red solid circle and dashed line), *S. Javiana* (green diamond and continuous line) at A. 15°C, B. 25°C, C. 30°C, and D. 37°C in nutrient solution for a duration of 21 days (n=3). Limit of detection ranged between 0.5 to 5 CFU/ml depending on the plating volume. Slopes from linear regression equations were used to calculate the decimal reduction values (*D*-values) of each bacterium at different temperature.

3. Results

3.1. Persistence of bacteria in hydroponic nutrient solution at each temperature

Statistical analysis indicated that a 3-way interaction effect between temperature, sampling day, and bacteria had a significant effect on bacterial persistence in nutrient solution ($p < 0.0001$), so interpretations cannot be made about main effects or 2-way interactions. The estimated mean log reduction of each bacterium at different temperatures in nutrient solution is illustrated in Figure 2.

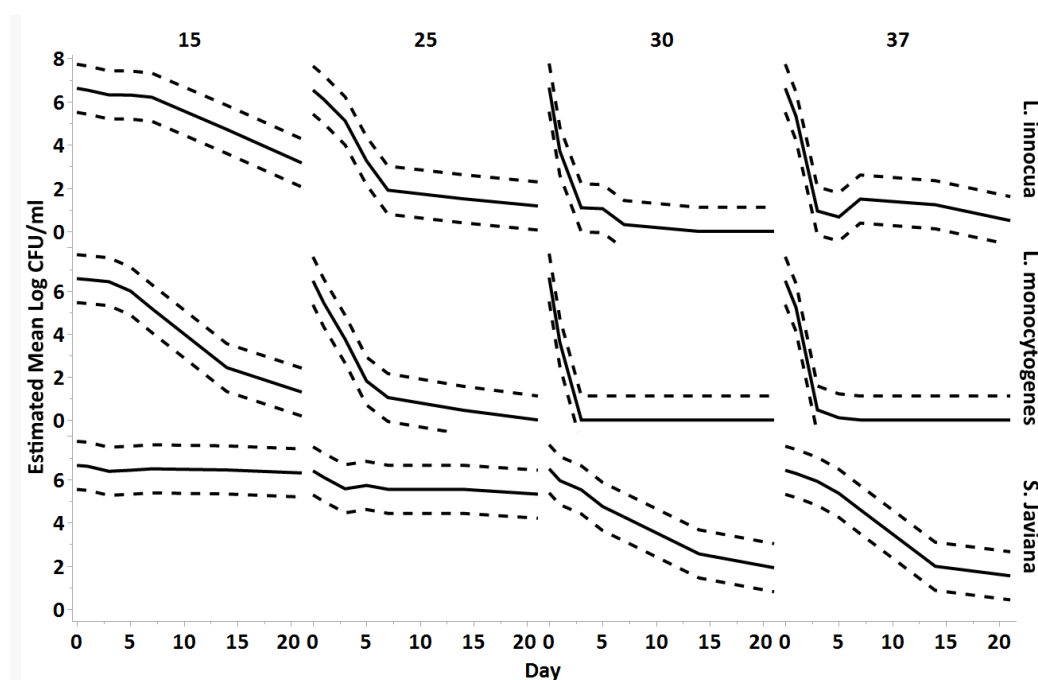


Figure 2. The continuous lines denote estimated mean log CFU/ml for all three bacteria recovered from hydroponic nutrient solution at different temperatures for 21 days of study. The dotted lines indicate 95% confidence intervals. Limit of detection (LOD) ranging between 0.5 to 5 CFU/ml.

At 15°C, there was no significant difference in the concentration of *L. innocua* and *L. monocytogenes* during day 0 to 7 of sampling. However, significant differences were observed on day 14 and 21 compared to other sampling days for both *L. innocua* and *L. monocytogenes* ($p \leq 0.0001$). There was little to no effect of temperature on the concentrations of *S. Javiana* recovered throughout 21 days of sampling. Moreover, *L. innocua*, *L. monocytogenes* and *S. Javiana* were detected throughout the 21 days of study at 15°C with an overall reduction of 2.1, 5.1, and 1.1 log CFU/ml, respectively (Table 2).

Table 2. Mean log (CFU/ml) (\pm standard deviation) of each bacterium in nutrient solution at 15°C for 21-day period.

Day	<i>L. innocua</i>	<i>L. monocytogenes</i>	<i>S. Javiana</i>
0	6.62 \pm 0.04	6.56 \pm 0.05	6.65 \pm 0.02
1	6.53 \pm 0.19	6.52 \pm 0.12	6.61 \pm 0.10
3	6.31 \pm 0.09	6.42 \pm 0.09	6.38 \pm 0.13
5	6.31 \pm 0.06	5.99 \pm 0.30	6.43 \pm 0.20
7	6.21 \pm 0.30	5.17 \pm 0.93	6.49 \pm 0.11
14	4.72 \pm 1.54	2.30 \pm 0.90	6.44 \pm 0.10
21	2.24 \pm 2.37	0.77 \pm 1.33	6.30 \pm 0.24

The data shown in the table are means \pm standard deviations for experiments performed in triplicates ($n=3$). LOD ranging between 0.5 - 5 CFU/ml depending on the volume plated.

At 25°C, after sampling on day 3, significant differences were observed in the concentrations of *L. innocua* and *L. monocytogenes* on day 5, 7, 14, and 21. ($p < 0.0001$). Whereas no significant differences were observed in bacterial recovery of *S. Javiana*, indicating that lower temperatures such as 15 and 25°C may have minimal effect on persistence of *S. Javiana* in hydroponic nutrient solution. Also, *L. innocua* and *S. Javiana* were detected throughout the 21 days of study with a ~ 5.5 and 1.2 log CFU/ml reduction, respectively (Table 3). However, *L. monocytogenes* was only detectable up to 7 days at 25°C with an estimated 6 log CFU/ml reduction.

Table 3. Mean log (CFU/ml) (\pm standard deviation) of each bacterium in nutrient solution at 25°C for 21-day period .

Day	<i>L. innocua</i>	<i>L. monocytogenes</i>	<i>S. Javiana</i>
0	6.53 \pm 0.15	6.46 \pm 0.18	6.40 \pm 0.21
1	6.11 \pm 0.79	5.44 \pm 1.23	6.10 \pm 0.26
3	5.11 \pm 1.26	3.39 \pm 2.94	5.57 \pm 0.43
5	3.21 \pm 1.84	1.81 \pm 1.72	5.72 \pm 0.49
7	1.91 \pm 1.71	0.53 \pm 0.92	5.55 \pm 0.38
14	1.51 \pm 1.32	ND	5.54 \pm 0.66
21	0.94 \pm 1.63	ND	5.32 \pm 1.17

The data shown in the table are means \pm standard deviations for experiments performed in triplicates (n=3). LOD ranging between 0.5 - 5 CFU/ml depending on the volume plated. ND = not detected.

At 30°C, after sampling on day 1 significant differences were noticed in concentrations of *L. innocua* and *L. monocytogenes* ($p < 0.0001$). Whereas significant difference in concentrations of *S. Javiana* were observed after sampling day 5 ($p < 0.0001$). Also *S. Javiana* survived throughout 21 days of the study with a ~ 4 log reduction, conversely *L. innocua* persisted only for 7 days, and *L. monocytogenes* persisted for one day in nutrient solution with a ~ 6 log reduction. This indicated that temperature had some influence on the survivability of *S. Javiana* in hydroponic nutrient solution over the period of 21 days.

Table 4. Mean log (CFU/ml) (\pm standard deviation) of each bacterium in nutrient solution at 30°C for 21-day period .

Day	<i>L. innocua</i>	<i>L. monocytogenes</i>	<i>S. Javiana</i>
0	6.65 \pm 0.07	6.62 \pm 0.10	6.49 \pm 0.24
1	3.71 \pm 3.29	3.60 \pm 1.56	6.35 \pm 0.06
3	1.09 \pm 1.89	ND	5.75 \pm 0.47
5	0.43 \pm 0.75	ND	5.00 \pm 0.16
7	ND	ND	4.56 \pm 0.66
14	ND	ND	2.42 \pm 2.43
21	ND	ND	1.62 \pm 2.30

The data shown in the table are means \pm standard deviations for experiments performed in triplicates (n=3). LOD ranging between 0.5 - 5 CFU/ml depending on the volume plated. ND = not detected.

At 37°C, a significant difference in *L. innocua* and *L. monocytogenes* were observed after sampling on day 1 ($p < 0.0001$), contrarily significant differences were observed in *S. Javiana* concentrations after sampling day 7 ($p < 0.0001$). Furthermore, *S. Javiana* persisted throughout the study period of 21 days with a reduction of 4.7 log CFU/ml, whereas *L. monocytogenes* and *L. innocua* survived in nutrient solution for 3 and 14 days, respectively, with a reduction of ~ 6 and 5.4 log CFU/ml indicating that higher temperatures may affect persistence of all three bacteria in the nutrient solution.

Table 5. Mean log (CFU/ml) (\pm standard deviation) of each bacterium in nutrient solution at 37°C for 21-day period .

Day	<i>L. innocua</i>	<i>L. monocytogenes</i>	<i>S. Javiana</i>
0	6.62 \pm 0.12	6.46 \pm 0.23	6.42 \pm 0.08
1	5.33 \pm 0.44	5.22 \pm 0.38	6.28 \pm 0.11
3	0.94 \pm 1.64	0.47 \pm 0.99	5.91 \pm 0.30
5	0.67 \pm 1.15	ND	5.36 \pm 0.35
7	1.50 \pm 1.32	ND	4.60 \pm 0.82
14	1.23 \pm 1.07	ND	1.98 \pm 2.33
21	ND	ND	1.38 \pm 2.40

The data shown in the table are means \pm standard deviations for experiments performed in triplicates (n=3). LOD ranging between 0.5 - 5 CFU/ml depending on the volume plated. ND = not detected.

3.2. Estimated decimal reduction values of bacteria in hydroponic nutrient solution by temperature (in days)

The D-values, or time to achieve a 1 log reduction, of *L. innocua*, *L. monocytogenes*, and *S. Javiana* in hydroponic nutrient solution were determined at 15, 25, 30, and 37°C (Table 6). As evidenced by pathogen log reduction at each temperature over time (Tables 2-5), the D-values of *S. Javiana* indicate greater stability in nutrient solution than *L. innocua* and *L. monocytogenes* at all temperatures. In addition, D-values differed across temperature for each bacteria evaluated. For instance, at 15°C and 25°C D-values for *S. Javiana* were estimated to be 82 and 26 days, respectively, compared to D-values of ~4 days at 30°C and 37°C. Similar trends were observed for *L. monocytogenes* and *L. innocua* across temperatures (Table 6).

Table 6. D-values indicate the estimated time (days) for 90% reduction of bacteria in hydroponic nutrient solution at different temperatures.

Temperature °C	Decimal Reduction Values (in Days)		
	<i>L. innocua</i>	<i>L. monocytogenes</i>	<i>S. Javiana</i>
15	6.08	3.59	82.60
25	4.26	3.06	26.30
30	1.64	0.47	4.54
37	4.47	0.89	4.13

4. Discussion

Water is the primary and essential component of hydroponic production. Fertilizer salts that are vital for the growth of leafy crops are generally mixed with water and used for hydroponic cultivation. Previous studies utilize various modified formulations of nutrient solution to evaluate the internalization and dissemination of enteric pathogens such as *Salmonella*, *L. monocytogenes*, Shiga toxin-producing *E. coli*, and human norovirus, within hydroponic systems for leafy greens production [8, 9, 13, 18, 19]. Unlike studies that have focused on investigating the internalization and propagation of human pathogens within leafy greens, the present study focuses on *S. Javiana*, *L. monocytogenes*, and *L. innocua* persistence in hydroponic nutrient solution without plants at different temperatures (15, 25, 30, and 37°C).

Hydroponic nutrient solutions are prepared using macro and micronutrients that are essential for leafy crop growth. Nutrient solution components include fertilizer salts such as calcium, copper, magnesium, sulfur, zinc, chlorine, and sodium [20]. Stokes *et al.* [21] discussed various bacteria and their dependency on specific nutrients including hydrogen, oxygen, phosphorus, carbon, sulfur, and potassium for their growth. The commonality of nutrients needed for hydroponic production of leafy greens and bacterial growth provides justification for the investigation of bacterial pathogen survival in nutrient solution and characterization of potential food safety risk.

The present study illustrated that bacterial persistence in nutrient solution can be temperature dependent. In a study by Xylia *et al.* [13], *S. Enteritidis* (6 log CFU/ml) was inoculated in hydroponic nutrient solution with different pH (5, 6, 7, and 8) and maintained at 21°C or 37°C for 21 h to determine bacterial growth kinetics. Xylia and coauthors observed about a 2 log CFU/ml (from 6 to 8 log CFU/ml) increase in *S. Enteritidis* in nutrient solution after 21 h at pH 5 and 6 regardless of temperature. This is contrary to the present study where an increase in *S. Javiana* was not observed after 24 h in nutrient solution (pH ~6) across all temperatures. Conversely, a minimum log reduction between 0.04 and 0.14 in *S. Javiana* was recorded across all temperatures after 24 h. The discrepancy in *Salmonella* growth kinetics between the present study and Xylia *et al.* [13] could be due to differences between *Salmonella* serovars, inoculum preparation (2x wash in PBS in the present study versus suspension in BHI), or the nutrient solution composition. Unfortunately, the composition of the nutrient solution was not provided by Xylia *et al.* [13].

Similarly, Shaw *et al.* [22] evaluated the persistence of bacterial pathogens in nutrient solution; however, the authors only considered microbial persistence at a single temperature over a 24 h period. Specifically, Shaw and co-authors inoculated spent nutrient solution (i.e., nutrient solution utilized over 4 weeks for growing basil in a DWC system), fresh nutrient solution, and distilled water separately with *E. coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* (STEC; cocktail including O26:H11, O45:H2, O103:H2, O111:H2, O121:H19, and O145NM), and *Salmonella* (cocktail of *S. Enteritidis* and *S. Typhimurium*). The inoculated nutrient solutions and water were held at 21°C, and samples were collected over the 24 h period. Shaw *et al.* (2016) reported an estimated 3 log CFU/ml increase for all pathogen types across all three solutions. *E. coli* O157:H7 bacterial load was higher in spent nutrient solution compared to water and fresh nutrient solution. Conversely, the concentrations of *Salmonella* and non-O157 STEC were higher in fresh nutrient solution and water compared to spent nutrient solution. This may be due to the presence of root associated microorganisms released into nutrient solution possibly leading to the suppression of *Salmonella* and non-O157 STEC in the spent nutrient solution used in the Shaw *et al.* (2016). Indeed, Dong *et al.* [22] reported the presence of a rich microbiome during hydroponic production of various types of microgreens and lettuce cultivars. This rich microbial community may impact pathogens due to nutrient competition or the presence of secondary metabolites secreted by the rhizosphere microbial community during hydroponic lettuce production [24, 25]. However, reasons for the inconsistent impact of the microbial community across pathogen types are unclear and warrant further investigation.

In another study [14], mineral nutrient solution (MNS) was used for the hydroponic cultivation of radish. One of their objectives was to inoculate MNS (pH 6) with eight pathogenic and/or spoilage bacteria (*Citrobacter freundii*, *Enterobacter* spp., *E. coli*, *Klebsiella oxytoca*, *Serratia grimesii*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*, and *L. monocytogenes*) and evaluate bacterial survival in MNS over a 28-day period. A single bacteria type was added to each system at 7 log CFU/ml. Due to diurnal variation, the average temperature of the MNS throughout production was 38.6°C (day) and 8.8°C (night). The study authors reported a microbial reduction of 3.1 to 5.1 log CFU/ml in MNS for all eight bacteria by the end of 28 days. Specifically, Settanni *et al.* [14] reported a 4-log reduction for *L. monocytogenes* after 28 days which is similar to the present study where *L. monocytogenes* decreased by up to 6 log CFU/ml across all temperatures within the 21-day period (Table 2 to 5).

In a study by Lopez-Galvez *et al.* [15], the microbiological quality of different irrigation water sources used during hydroponic production of tomatoes in greenhouses was analyzed. The irrigation water types included reclaimed water with or without fertilizer solution and surface water with or without fertilizer solution. Five separate fertilizer solutions were used during hydroponic production: monopotassium phosphate (F1), potassium nitrate (F2), calcium nitrate (F3), microelements solution (F4), and nitric acid (F5). The authors also collected drainage water samples (irrigated with reclaimed water or surface water) from growth substrates used during production. Water samples were collected over 13 weeks and analyzed for indicator bacteria (generic *E. coli* and *Listeria* spp.) and pathogenic bacteria (STEC, *L. monocytogenes*, and *Salmonella* spp.). The authors reported higher levels of *Listeria* spp. in both surface and reclaimed water with fertilizer solutions compared to water sources without fertilizers along with drainage water from the growth substrates. As a result, individual fertilizer solutions were then analyzed for the presence of *Listeria* spp., and elevated counts (4.5 log CFU/100ml) within the potassium nitrate solution were observed; however, none were positive for *L. monocytogenes* by real time PCR. Meanwhile, 8 out of 104 water samples were presumptively positive for *Salmonella* including reclaimed water with and without fertilizer, surface water with and without fertilizer, and drainage water from the rockwool growth substrate. During the study by Lopez-Galvez *et al.* [15], the ambient temperature of the greenhouse was between 15.0°C and 29.1°C, and electrical conductivity and pH were maintained at ≤ 2 dS/m and 5.5, respectively, within the hydroponic

systems. However, it is difficult to determine the impact of temperature on bacterial persistence since the internal temperature of the nutrient solution within hydroponic systems were not recorded over the 13-week period. Overall, Lopez-Galvez and co-authors [15] demonstrate the potential for fertilizer solutions to introduce microbial contamination within a hydroponic system via recirculating nutrient solutions.

The recent outbreak and recall associated with hydroponic production of leafy greens has created greater awareness regarding the associated food safety challenges within CEA [3, 26]. The present study indicates that temperature may be an important factor in the persistence of human pathogens in hydroponic nutrient solution during production. Specifically, the data demonstrate that temperature has a greater impact on the persistence of *L. monocytogenes* and *L. innocua* over a 21-day period compared to *S. Javiana*, specifically at lower temperatures such as 15 and 20°C. Based on these data, it could be anticipated that *Salmonella* might pose a greater food safety risk during hydroponic production of leafy greens when compared to *Listeria* spp. As a result, there may be a higher probability of internalization of *Salmonella* in leafy greens grown in hydroponic systems.

Author Contributions: **Gayatri Dhulappanavar:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Kristen E. Gibson:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Funding: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-68008-31559 from the USDA National Institute of Food and Agriculture.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We thank Dr. Ryan W. Dickson for helping us with Hoagland's nutrient solution formulation. We appreciate Dr. Christopher A. Baker and Dr. Wenjun Deng for their support and valuable inputs. We also thank Kevin Thompson for his assistance with data analysis.

Conflicts of Interest: No conflict of interest declared.

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