academicJournals

Vol. 10(15), pp. 528-534, 21 April, 2016
DOI: 10.5897/AJMR2015.7784
Article Number: DAECED558170
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Biocontrol of *Salmonella* Typhimurium growth in tomato surface by bacteriophage P22

Osvaldo López-Cuevas[1], Nohelia Castro-del Campo[1], Karina Ramirez[2] and Cristóbal Chaidez[1]*

[1]Laboratorio Nacional para la Investigación en Inocuidad Alimentaria, Centro de Investigación en Alimentación y Desarrollo A. C. Carretera a El dorado Km. 5.5, Campo El Diez, Culiacán, Sinaloa 80110, Mexico.
[2]División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Culiacán. Calle Juan de Dios Bátiz, No. 310 pte. Colonia Guadalupe, Culiacán, Sinaloa 80220, México.

Received 30 September, 2015, Accepted 18 March, 2016

The aim of this research was to evaluate the effect of bacteriophage P22 on *Salmonella* Typhimurium in artificially inoculated tomato surface, under simulating condition of storage (10°C) and marketing (20°C) during seven days. First, we demonstrated the *in vitro* ability of phage P22 to reduce host population density to undetectable levels two hours after inoculation; however, *S.* Typhimurium was able to regrow up to 5.5 Log_{10}CFU/ml after 24 h. *S.* Typhimurium-inoculated tomatoes stored at 10°C and exposed to P22 exhibited a mean bacterial reduction of 3.02 Log_{10}CFU/tomato, while those stored at 20°C showed a mean reduction of 0.7 Log_{10}CFU/tomato at day seven. Although phage P22 was able to reduce *S.* Typhimurium on tomato surface, the *in vitro* results indicate that a larger initial concentration of phages is required in order to ensure sustained inactivation of *S.* Typhimurium.

Key words: Biocontrol, bacteriophages, *S.* Typhimurium, tomato.

INTRODUCTION

The number of foodborne illness outbreaks linked to fresh produce has increased in the last years (Beuchat, 1996; Lynch et al., 2009). Pathogens most commonly associated with the consumption of fresh produce are viruses, bacteria and protozoa (DeWaal et al., 2007). These microorganisms cause approximately 76 million new cases of food-related illness in the United States each year, resulting in 325,000 hospitalizations and 5,000 deaths, while the economic cost generated by these events is around 152 billion dollars (Scharff, 2010). Consumption of tomatoes and peppers has been recently responsible of foodborne outbreaks caused by *Salmonella* serovars, which have involved from sporadic cases to multistate outbreaks, implicating severe problems in public health (Hedberg et al., 1999; Behravesh et al., 2011). Fresh produce contamination can occur from the field to the table; according to Lynch et al. (2009) the probability of contamination increases in specific production steps, including field work, packinghouse, and also during final processing in the

*Corresponding author. E-mail: chaqui@ciad.mx. Tel: (52) 667 7605536. Fax: (52) 667 7605537.

kitchen. *Salmonella* is the main bacteria responsible for fresh produce outbreaks (Hanning et al., 2009; Fatica and Schneider, 2011), being the leading cause of hospitalization and deaths with a 35 and 28%, respectively (González and Rojas, 2005). Outbreaks of *Salmonella* Saint Paul occurred in 2008 and 2013 in the United States sickened 1,442 people in 43 states, and 81 people in 18 states, respectively. These outbreaks were associated (as suspects) with the consumption of jalapeño pepper and cucumber (CDC, 2008, 2013a). The occurrence of outbreaks evidences the failure of physical and chemical interventions methods (Parish et al., 2003).

In this regard, the use of bacteriophages as biocontrol, individually or combined with chemical products, has been proposed (Xu, 1999). Previous reports have indicated the efficacy of bacteriophages to eliminate *Salmonella* from meat, fresh produce and processed foods. Leverentz et al. (2001) applied a mixture of 4 lytic bacteriophages (SCPLX-1) specific for *Salmonella* Enteritidis on pre-cut melons and apples stored at temperatures of 5, 10 and 20°C in a period of 168 h achieving bacterial reductions of 3.5 Log_{10}CFU/melon at 5 and 10°C, and 2.5 Log_{10}CFU/melon at 20°C. Another study showed the effect of bacteriophages over *S.* Enteritidis suppression in Cheddar cheese produced from raw and pasteurized milk and stored at 8°C; *S.* Enteritidis did not survive in pasteurized milk cheese after 89 days in the presence of the phage. However, *S*. Enteritidis was able to survive around 50 CFU/g in raw milk cheese containing phage even after 99 days of storage (Modi et al., 2001). Waseh et al. (2010) orally administered P22 phage tailspike protein in chicken infected with *Salmonella* and found a reduction in bacterial colonization. These findings coincide with the ability of bacteriophages to reduce bacterial populations from foods.

Considering that *S. enterica* is a major foodborne pathogen generally involved in fresh produce-borne outbreaks (CDC, 2013b) and that tomatoes have been implicated in recent illness cases (CDC, 2013c), in this study we determined the efficacy of bacteriophage P22 to reduce *S. enterica* subsp. *enterica* serovar Typhimurium on tomato surface in order to demonstrate the feasibility of successful biocontrol against *Salmonella*.

MATERIALS AND METHODS

Bacterium inoculum

Host strain: *Salmonella* Typhimurium LT2 (ATCC® 19585™) inoculum was obtained by the addition of a loop of the bacterium to 6 L of trypticase soy broth (TSB, Bioxon, México) and incubated for 24 h at 37°C. Bacteria were concentrated to 500 ml using an ultra-filtration system (Hernandez-Morga et al., 2009). The bacterial concentrate was then centrifuged at 13,800×*g* for 10 min at 4°C (Thermo IEC Multi RF, 8466, USA). The pellet was washed two times with 100 ml of sterile monobasic phosphate buffered solution (PBS) [0.01 M, pH 7.2, Sigma-Aldrich, Inc. Saint Louis, MO, USA] and resuspended in 6 L of PBS. The cell suspension was adjusted to a final OD_{600} of 1 (Eppendorf, Hamburg, Germany), according to Leverentz et al. (2001). The bacterial inoculum was quantified using the spread plate technique to obtain a final concentration of viable bacteria of 10^9 CFU/ml (APHA, 1998).

Bacteriophage P22 propagation

Bacteriophage P22 was kindly provided by Dr. Charles P. Gerba (University of Arizona). In order to propagate the bacteriophage, five millilitre of the P22 stock and 50 ml of *S.* Typhimurium (10^9 CFU/ml) were added to two litters of sterile PBS, pH 7.2 (Whichard et al., 2003), and incubated in a water bath overnight at 37°C and 110 rpm (Shak R Bath 3580 R, Lab-Line Instruments, Inc, USA). The mixture was then concentrated to 500 ml with an ultra-filtration system adapted from Hill et al. (2005) and Hernández-Morga et al. (2009), followed by centrifugation at 13,800×*g* for 10 min at 4°C. The supernatant was filtered through sterile cellulose acetate 0.22 µm membrane filters (Corning®, NY, USA). The bacteriophage titer was determined by the soft agar overlay technique (APHA, 1998). The final bacteriophage concentration was 10^{11} PFU/ml.

In vitro assay for bacterial biocontrol

In order to determine the ability of bacteriophage P22 to lyse *S.* Typhimurium (ATCC® 19585™), an experiment in liquid culture at 37°C for 48 h was performed. One colony of *S.* Typhimurium was grown in 10 ml of TSB at 37°C overnight. Then, aliquots of 1 ml were individually transferred to six flasks, each containing 49 ml of pre-warmed TSB and incubated at 37°C until the OD_{600} was 0.5 (ca. 1.5×10^8 CFU/ml). The bacteriophage P22 was individually inoculated into the bacterial cultures at a theoretical MOI of 100 and 1000 (in duplicated); additionally, two flasks were not inoculated (Control). Bacterial titer was measured in Hektoen agar plates at 2, 4, 6, 8, 12, 24 and 48 h after bacteriophage inoculation. The experiment was performed in duplicate.

Tomato preparation

Roma saladette tomatoes under physiological maturity were obtained from the greenhouse of the Centro de Investigación en Alimentación y Desarrollo, Culiacán Station. Homogeneous size and free of mechanical damage tomatoes were selected and disinfected by immersion into a sodium hypochlorite solution (Fermont, México) adjusted at 300 mg/L for 10 min. Then tomatoes were immersed in a 5% sodium thiosulfate solution (Fermont, México) for 10 min, and finally rinsed with sterile purified water and stored at 4°C for 12 h prior use. Tomatoes were evaluated for the absence of microbial contaminants after the disinfection procedure.

Biocontrol of *S.* Typhimurium on tomato surface

For this purpose, the immersion method was performed as previously described by Chaidez et al. (2007) with some modifications. Briefly, 72 tomatoes were divided into three groups: the first group consisted of 24 *S.* Typhimurium -inoculated tomatoes sprayed with bacteriophage P22; the second group had 24 *S.* Typhimurium -inoculated tomatoes sprayed with sterile PBS; and the third group had 24 tomatoes sprayed with P22 alone (control). For the first two groups, tomatoes were immersed for 15 min in 6 L of bacterial suspension (10^9 CFU/ml) with constant agitation, whereas the last group (control) was not inoculated with anything. Then, all of tomatoes were dried for 1 h in two parallel sterile glass rods located in a biosafety cabinet and stored in sterilized bags (Ziploc®, SC Johnson USA) at 4°C until use (Lang et al., 2004).

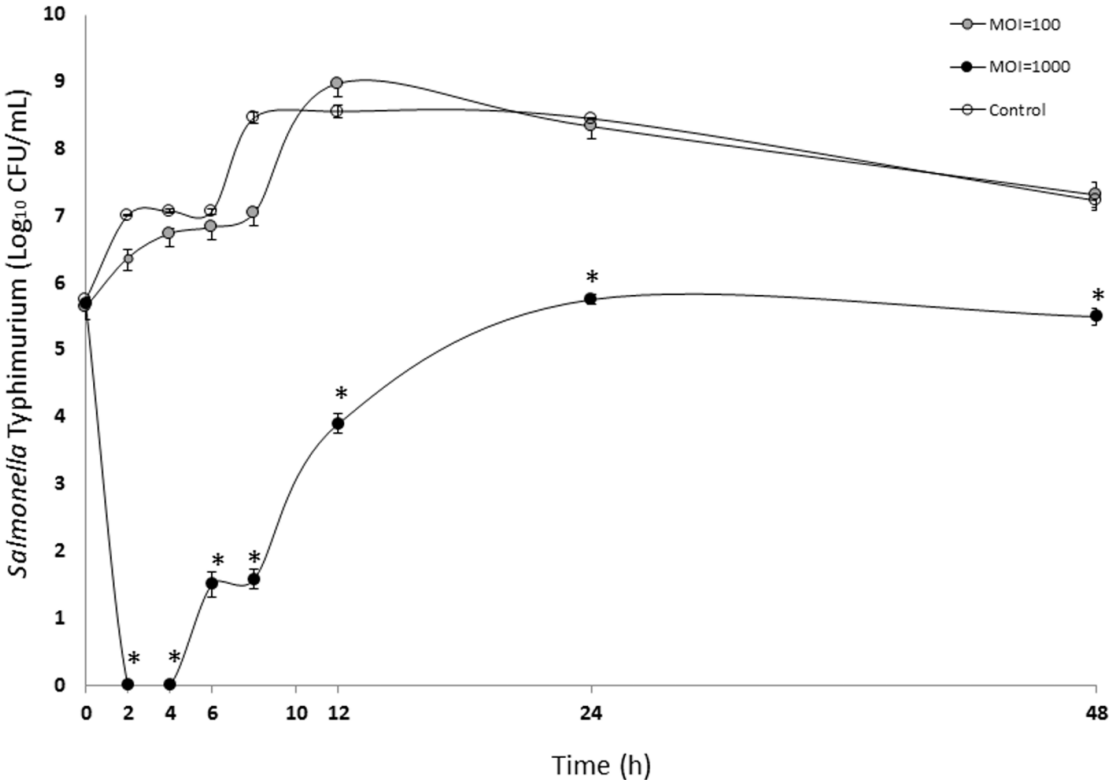

Figure 1. *In vitro Salmonella* infection by bacteriophage P22. Bacteria were infected with phage P22 at MOI of 100 and 1, 000 at 37°C for 48 h. Data represents mean Log_{10}CFU/ml ±SE. Significant differences (* *P<0.005*) compared with the non-infected control group are indicated.

Bacteriophage P22 (10^{11} PFU/ml) was sprayed onto *S.* Typhimurium -inoculated and control tomatoes for 45 s from a distance of 45 cm with a manual sprayer (SPRAY-33, Truper®, México) to simulate packinghouse spray disinfecting conditions. Following bacteria and bacteriophage inoculation, one set of each group was stored at 10°C and another set was stored at 20°C. Tomatoes were analyzed every 24 h for 7 days. Two independent experiments were performed for each temperature with 3 replicates per experiment.

Recovery and enumeration of *S.* Typhimurium and bacteriophage P22

Treated tomatoes (n=3), as described above, were randomly chosen and evaluated every 24 h for 7 d for bacteria and phage recovery. Each tomato was placed in a sterile plastic bag containing 100 ml of PBS and manually rinsed by rubbing for 1 min to remove *S.* Typhimurium and bacteriophage from the surface (Hirotani et al., 2002). Samples were serially diluted and plated in duplicate on XLD agar (Bioxon, México). Plates were incubated at 37°C and enumerated after 24 h. Bacteriophage concentration was determined using the soft agar overlay as previously described (APHA, 1998). Data was expressed as Log_{10}CFU/tomato and Log_{10}PFU/tomato, for bacterial and viral quantification, respectively.

Statistical analysis

The survival of *S.* Typhimurium on tomato surface was determined by a design of two blocks completely randomized. The persistence of bacteriophage P22 on tomato surface was analyzed with a three complete randomized block design. Efficiency of bacteriophage P22 for *S.* Typhimurium reduction on tomato surface was measured with a three complete randomized block design. Analysis of variance and multiple mean comparisons were conducted through the Tukey test. Differences with P<0.05 were considered significant. Data analysis was performed using MINITAB version 14 (Minitab Inc., State College, PA).

RESULTS

In vitro assay for bacterial biocontrol

The bacteria/bacteriophage challenge in a liquid culture is a passive approach (*in vitro*) to demonstrate the lytic effect of phages against target bacteria. In this case, high multiplicity of infection (MOI) of 100 and 1000 were used to ensure a bacteriophage/bacteria ratio in the system sufficient to infect all target bacteria by at least one phage particle, and induce bacteria lysis in a short period of time. This approach may predict the challenge behavior *in vivo*. Kinetics of *Salmonella* growth is shown in Figure 1. *Salmonella* infected with P22 at the highest MOI ratio of 1,000 showed undetectable levels of bacteria between two and four hours after infection; nonetheless,

measurable bacteria were observed after six hours with a peak value of 5.5 Log_{10}CFU/ml at 48 h after infection (Figure 1, P<0.001). Meanwhile, at the MOI of 100, bacteriophage P22 was unable to significantly reduce *Salmonella* growth, however somewhat delay was observed in bacteria growth between two and four hours after infection (Figure 1, P<=0.005). The bacterial control concentration was 7.48±0.2 Log_{10}CFU/ml after 48 h of incubation. These results indicate that phage P22 is able to lyse *Salmonella* Typhimurium at a higher MOI, however *Salmonella* regrowth at low and high MOI suggests that *Salmonella* may develop Bacteriophage Insensitive Mutants (BIM´s) or a lysogenic cycle due to experimental conditions.

S. Typhimurium survival on tomato surface

To evaluate *Salmonella* survival on tomato surface, tomatoes were inoculated with *S.* Typhimurium and stored at 10 and 20°C for seven days. Tomatoes maintained at 10°C exhibited the lowest *Salmonella* concentrations during storage, with a mean concentration of 6.29 Log_{10}CFU/tomato and maximum/minimum concentrations of 6.5 and 5.83 Log_{10}CFU/tomato, respectively, at day seven. On the other hand, tomatoes stored at 20°C exhibited higher *S.* Typhimurium concentrations than tomatoes stored at 10ºC showing an increasing tendency as the contact time increased (from 7.1 Log_{10} CFU/tomato at time zero to 8.32 Log_{10} CFU/tomato at day seven), with a mean concentration of 7.69 Log_{10}CFU/tomato, which suggest a relationship between temperature and contact time.

Bacteriophage P22 survival on tomato surface

Phage survival was assessed on tomato surface sprayed with P22 alone or in presence of *S.* Typhimurium and stored at 10 and 20°C during the seven days. Both conditions showed decrease on phage survival after the storage period. In tomatoes simultaneously inoculated with *Salmonella* and P22 at 10°C, P22 was reduced up to 7.23 Log_{10} PFU/tomato (3.79 Log_{10} PFU/tomato reduction), as compared to initial phage concentration (11.02 Log_{10}PFU/tomato); on the other hand, tomatoes inoculated only with P22, phage was reduced 4.03 Log_{10} PFU/tomato between day 0 and day seven after inoculation.

High levels of P22 were detected when tomatoes were sprayed with *S.* Typhimurium along with P22 and stored at 20°C. However, a reduction of 3.24 Log_{10}PFU/tomato was observed on day seven with a final concentration of 8.36 Log_{10}PFU/tomato. In contrast, tomatoes inoculated only with P22 showed a reduction of 4.11 Log_{10}PFU/tomato with a final concentration of 6.9 Log_{10}PFU/tomato on day seven. These results show that phage had a reduction in their concentration, regardless of the experimental condition in all cases.

Biocontrol of *S.* Typhimurium on tomato surface

Bacteriophage P22 lytic effect on *S.* Typhimurium inoculated onto tomato surface stored at 10 and 20°C during seven days is shown in Figure 2. Control groups for both temperatures where inoculated only with *Salmonella.* P22 treated-tomatoes stored at 10°C registered a constant decrease of *S.* Typhimurium concentration (Figure 2B). The highest reduction was detected on day seven with 3.02 Log_{10}CFU/tomato, compared to the baseline concentration, while the mean reduction concentration during the seven-day experiment was 1.38 Log_{10}CFU/tomato. P22 treated-tomato and stored at 20°C presented a mean bacterial reduction of 0.7 Log_{10}CFU/tomato seven days after inoculation (Figure 2C). Control groups showed higher *S.* Typhimurium concentrations as compared to tomatoes that received bacteria and P22 (Figure 2B and C).

DISCUSSION

The use of bacteriophages to reduce contamination of *S.* Typhimurium has several advantages over the use of chemical sanitizers. Firstly, bacteriophages are prokaryotic cells specific and, therefore, phages possess no harm to plants and/or animal cells. Secondly, within prokaryotic cells, bacteriophages will only lyse target bacteria, leaving fresh produce microbiota intact (Ackermann and DuBow, 1987; Gill and Abedon, 2003).

However, one of the biggest concerns of using bacteriophages as biocontrol is that they might shift from lytic to lysogenic cycle. During this cycle, phage inserts its nucleic acid into the bacterium and may transfer resistance or virulence genes (if phage contained), resulting in more virulent and resistant bacteria. However, reports have suggested that bacteriophage P22 showed low frequency of lysogenization cycle, which may be attributed to *Salmonella*'s deficiency of adenilatecyclase (*cya* gene) or to the receptive protein (*crp* gene) production (Hong et al., 1971). Furthermore, Steinberg and Gough (1976) demonstrated that *S.* Typhimurium infection by wild type phage P22 causes a lytic response to low multiplicities of infection and a lysogenic response to high multiplicities of infection (MOI >5 more than 90% are lysogenized). Merril et al. (1996) reported the potential use of a mutated variant of P22 as therapeutic agent for bacteremia. In our results, plaques developed by P22 were clear and with 0.5-0.6 mm in diameter (data not shown), which suggests that P22 may have developed lytic cycle in this experimentation (Hong et al., 1971).

The use of high phage MOI has been described to lead

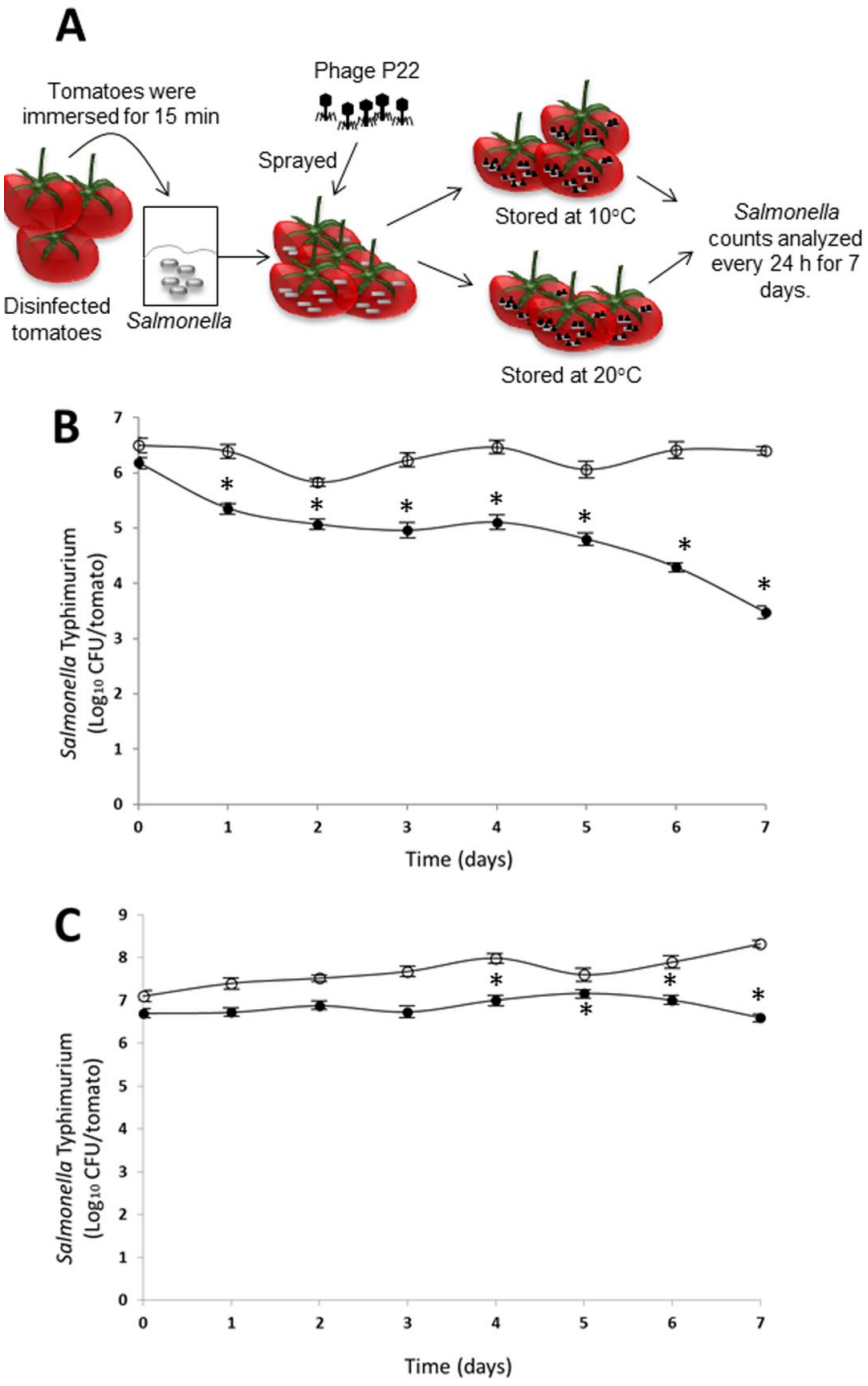

Figure 2. *Salmonella* reduction by bacteriophage P22 on tomato surfaces. (A) Schematic representation illustrating the biocontrol of *S.* Typhimurium on tomato surface. Tomatoes were sprayed with *Salmonella* in the presence of P22 (closed circles) or with *Salmonella* alone (open circles) and stored at 10°C (B) and 20°C (C) during seven days. Data represents mean Log_{10}CFU/tomato ±SE. Significant differences (*P<0.002) compared with the non-infected control group are indicated.

an early bacterial lysis because high virion particles are adsorbed on the bacterial surface and cause the weakened of the cell wall and consequently bacterial lysis, without bacteriophage particles production. Abedon (2011) describes this phenomenon as “Lysis from without (LO)”, which has been demonstrated in various bacteriophages, such as like T4 phages. Although not all phages develop LO, bacterial lysis at high MOI in our

results would be explained by the LO phenomenon.

The *in vitro* infection assay, showed that P22 was able to reduce *Salmonella* to undetectable levels 4 h after phage inoculation; however, after this period the bacterium was able to re-grow, reaching levels close to the control. This effect is most likely associated with the low proportion of P22 in a lysogenic state, which allows *Salmonella* suppression at initial stages; however, lysogenized bacteria could re-grow during long-term incubation (Gama et al., 2013). It is also important to emphasize that enrichment step (rich culture medium and optimal temperature) provide ideal conditions for bacterial growth, whereas *in vivo* conditions might present low nutrient availability or non-permissive temperatures for *Salmonella* growth.

According to our data, *S.* Typhimurium (without P22 treatments) was able to survive in tomato surface at storage temperature of 10°C for at least seven days, showing a marginal reduction of 0.67 Log_{10} CFU/tomato at the end of experimentation. Contrary to what it was observed to tomatoes stored at 20°C, where an increased concentration of *Salmonella* (1.22 Log_{10} CFU/tomato) was observed at seventh day compared to day zero, showing an increasing tendency as increases time; which suggests that *Salmonella* may be growing under these conditions. Other studies also described an increase in bacterial populations when fruits were stored at high temperatures (20 and 25°C) compared to low temperatures (10 and 4°C) where they recorded reductions up to 2 Log_{10} on fresh-cut fresh produce (Leverentz et al., 2001; Castro-del Campo et al., 2004). All microorganisms have an optimal growth temperature at which they exhibit their highest growth rate.

In this sense, the increased concentration of *Salmonella* could be attributed to the mesophilic nature of the bacteria. Zhuang et al. (1995) suggested that viability of *Salmonella* on the surface of tomatoes at 10°C might be a potential survival temperature during transport and storage prior to ripening and consumption; and even more, our results showed that at 20°C (marketing conditions) *Salmonella* was able to grow and increases its concentration, what hypothetically could represent a risk of salmonellosis outbreak if a scenery similar occurs in real conditions; therefore, suitable storage temperature of fruits and vegetables is fundamental to maintain low microbial levels and prevent pathogenic microorganism's growth (Leverentz et al., 2001).

In this investigation, bacteriophage P22 showed a positive lytic effect over *S.* Typhimurium, achieving a maximum reduction of 3.02 Log_{10}CFU/tomato in seven days at 10°C, demonstrating its effectiveness to reduce populations of a pathogenic microorganism, as reported by Sklar and Joerger (2001); Modi et al. (2001); Pao et al. (2004) and López-Cuevas et al. (2012). *Listeria monocytogenes* was reduced in high concentrations (5.7 Log_{10}CFU/ml) in fresh-cut honeydew melon, and higher reductions were achieved through a combination of bacteriophages and bacteriocins (Leverentz et al., 2003). Several studies explore bacteria reductions in fresh-cut produce with phages. For the best of our knowledge, the present study is one of the few studies to be conducted in whole fruit.

This research provides data about the effectiveness of the use of bacteriophages to reduce or eliminate pathogenic microorganisms present on fresh produce. The US-EPA determined the reasonable standard of efficiency of a disinfectant in a fresh produce surface that at least should achieve a 2 Log_{10}CFU reduction of the pathogen population (EPA, 1997). Thus, the use of bacteriophages is a natural alternative, which could benefit both conventional and organic agriculture.

Conclusion

This research data suggests that bacteriophage P22 would provide a protective effect to fresh produce through the production chain as a novel, environmentally safe alternative to control *Salmonella enterica* subsp. *enterica* on fresh produce.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank the National Food Safety Laboratory Research personnel (LANIIA-CIAD). We also thank Célida Martinez-Rodríguez for her technical assistance.

REFERENCES

Abedon ST (2011). Lysis from without. Bacteriophage 1(1):46-49.

Ackermann HW, DuBow MS (1987). Practical applications of bacteriophages. In Viruses of prokaryotes: general properties of bacteriophages. CRC Press, Boca Raton, Fla., USA, pp. 143-158.

American Public Health Association [APHA] (1998). Standard methods for the examination of water and wastewater, 20th edition. American Public Health Association, Washington, DCAPHA.

Behravesh CB, Rajal K, Mody MD, Jungk J, Gaul L, Redd JT, Chen S, Cosgrove S, Hedican E, Sweat D, Chávez-Hauser L, Snow SL, Hanson H, Nguyen T, Sodha SV, Boore AL, Russo E, Mikoleit M, Theobald L, Gerner-Smidt P, Hoekstra RM, Angulo FJ, Swerdlow DL, Tauxe RV, Griffin PM, Williams IT (2011). 2008 Outbreak of *Salmonella* Saintpaul Infections Associated with Raw Produce. New. Engl. J. Med. 364(10):918-927.

Beuchat LR (1996). Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59(2):204-216.

Castro-del Campo N, Chaidez QC, Rubio CW, Valdez TJV (2004). Sobrevivencia de *Escherichia coli* y *Staphylococcus aureus* en frutos mínimamente procesados. Rev. Cub. Salud Pública. 30(1):83-86.

CDC (2008). Outbreak of *Salmonella* serotype *Saintpaul* infections associated with multiple raw produce items - United States. Available from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5734a1.htm.

Accessed 30/10/2014.
CDC (2013a). FDA Investigates Multistate Outbreak of *Salmonella* Saintpaul linked to cucumbers supplied by Daniel Cardenas Izabal and Miracle Greenhouse. Available at: http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm349461.htm. Accessed 13/11/ 2014.
CDC (2013b). Reports of selected *Salmonella* outbreak investigations. Available at. http://www.cdc.gov/salmonella/outbreaks.html. Accessed 13/11/2014.
CDC (2013c). Multistate Outbreak of *Salmonella* Typhimurium Infections Linked to Tomatoes (Final Update). Available at: http://www.cdc.gov/salmonella/typh2006/index.html. Accessed 13/11/2014.
Chaidez QC, López J, Vidales J, Castro-del Campo N (2007). Efficacy of chlorinated and ozonated water in reducing *Salmonella typhimurium* attached to tomato surfaces. Int. J. Environ. Health Res. 17(4): 311-318.
DeWaal CS, Barlow K, Alderton L, Jacobson MF (2007). Outbreak alert closing the gaps in our federal-food safety net. Center for Science in the Public Interest. Available from: http://www.cspinet.org/foodsafety/outbreak_alert.pdf. Accessed 03/12/2014.
Environmental Protection Agency [EPA] (1997). A Set of scientific issues being considered by the agency in connection with the efficacy testing issues concerning public health antimicrobial pesticides. Scientific Advisory Panel September 1997 Meeting Final Report. Available at: http://www.epa.gov/scipoly/sap/1997september/finalsep.htm. Accessed 03/10/2014.
Fatica MK, Schneider KR (2011). *Salmonella* and produce: Survival in the plant environment and implications in food safety. Virulence 2(6):573-579.
Gama JA, Reis AM, Domingues I, Mendes-Soares H, Matos AM, Dionisio F (2013). Temperate Bacterial Viruses as Double-Edged Swords in Bacterial Warfare. PLoS ONE 8(3):e59043.
Gill JJ, Abedon TS (2003). Bacteriophage ecology and plants. APSnet Feature. Available at: http://www.apsnet.org/online/feature/phages/abedon.pdf. Accessed 21/12/2014.
González FM, Rojas HR (2005). Foodborne diseases and PCR: Disease Prevention and diagnosis. Enfermedades transmitidas por Alimentos y PCR: Prevención y diagnóstico. Salud Públ. Méx. 47(5): 388-390.
Hanning IB, Nutt JD, Ricke SC (2009). Salmonellosis Outbreaks in the United States Due to Fresh Produce: Sources and Potential Intervention Measures. Foodborne Pathog. Dis. 6(6):635-648.
Hedberg CW, Angulo FJ, White KE, Langkop CW, Schell WL, Stobierski MG, Schuchat A, Besser JM, Dietrich S, Helsel L, Griffin PM, McFarland JW, Osterholm MT (1999). Outbreaks of salmonellosis associated with eating uncooked tomatoes: Implications for public health. Epidemiol. Infect. 122:385-393
Hernandez-Morga J, Leon-Felix J, Peraza-Garay F, Gil-Salas BG, Chaidez C (2009). Detection and characterization of hepatitis A virus and Norovirus in estuarine water samples using ultrafiltration - RT-PCR integrated methods. J. Appl. Microbiol. 106(5):1579-1590.
Hill VR, Polaczyk AL, Hahn D, Narayanan J, Cromeans TL, Roberts JM, Amburgey JE (2005). Development of a rapid method for simultaneous recovery of diverse microbes in drinking water by ultrafiltration with sodium polyphosphate and surfactants. Appl. Environ. Microbiol. 71(11):6878-6884.
Hirotani H, Naranjo J, Moroyoqui PG, Gerba CP (2002). Demonstration of Indicator Microorganisms on Surface of Vegetables on the Market in the United States and Mexico. J. Food Sci. 67(5):1847-1850.
Hong J, Smith G, Ames B (1971). Adenosine 3':5'-Cyclic Monophosphate Concentration in the Bacterial Host Regulates the Viral Decision between Lysogeny and Lysis. Proc. Natl. Acad. Sci. USA 68(9):2258-2262.
Lang MM, Harris LJ, Beuchat LR (2004). Evaluation of inoculation method and inoculum drying time for their effects on survival and efficiency of recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on the surface of tomatoes. J. Food Prot. 67(4):732-741.
Leverentz B, Conway W, Alavidze Z, Janisiewicz W, Fuchs Y, Camp M, Chighladze Y, Sulakvelidze E (2001). Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: A model study. J. Food Prot. 64(8):1116-1121.
Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, Saftner R, Sulakvelidze A (2003). Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Appl. Environ. Microbiol. 69(8):4519-4526.
López-Cuevas O, Castro-del Campo N, León-Félix J, Valdez-Torres, Chaidez C (2012). Evaluation of bacteriophage Av-08 for simultaneous biocontrol of *Salmonella* Montevideo and *Escherichia coli* O157:H7 in experimentally contaminated chicken skin. J. Food Saf. 32:305-310.
Lynch MF, Tauxe RV, Hedberg CW (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: Risks and opportunities. Epidemiol. Infect. 137:307-315.
Merril C, Biswas B, Carltont R, Jensen N, Creed J, Zullo S, Adhyat S (1996). Long-circulating bacteriophage as antibacterial agents. Proc. Natl. Acad. Sci. 93(8):3188-3192.
Modi R, Hirvi Y, Hil A, Griffiths MW (2001). Effect of phage on the survival of *Salmonella enteritidis* during manufacture and storage of cheddar cheese made from raw and pasteurized milk. J. Food Prot. 64(7):927-33.
Pao S, Randolph SP, Westbrook EW, Shen H (2004). Use of bacteriophages to control *Salmonella* in experimentally contaminated Sprout Seeds. J. Food Sci. 69(5):127-130.
Parish ME, Beuchat LR, Suslow TV, Harris LJ, Garret EH, Farber JN, Busta FF (2003). Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. Comprehens. Rev. Food Sci. Food Saf. 2(1):161-173.
Scharff RL (2010). Health-Related Costs From Foodborne Illness In The United States. For the Produce Safety Project at Georgetown University. Available at: http://publichealth.lacounty.gov/eh/docs/ReportPublication/HlthRelatedCostsFromFoodborneIllinessUS.pdf. Accessed 21/09/2015.
Sklar IB, Joerger RD (2001). Attempts to Utilize Bacteriophage to Combat *Salmonella enterica* serovar Enteritidis Infection in Chickens. J. Food Saf. 21(1):15-29.
Steinberg B, Gough M (1976). Bacteriophage P22 lysogenises efficiently at high multiplicities of infection because *Salmonella typhimurium* DNA synthetic capacity is limited. Nature 263:54-56.
Waseh S, Hanifi-Moghaddam P, Coleman R, Masotti M, Ryan S, Foss M, MacKenzie R, Henry M, Szymanski CM, Tanha J (2010). Orally Administered P22 Phage Tailspike Protein Reduces *Salmonella* Colonization in Chickens: Prospects of a Novel Therapy against Bacterial Infections. PLoS ONE 5(11):13904.
Whichard JM, Sriranganathan N, Pierson FW (2003). Suppression of *Salmonella* growth by wild-type and large-plaque variants of bacteriophage felix 01 in liquid culture and on chicken frankfurters. J. Food Prot. 66(2):220-225.
Xu L (1999). Use of ozone to improve the safety of fresh fruit and vegetables. Food Technol. 53(10):58-62.
Zhuang RY, Beuchat LR, Angulo FJ (1995). Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. Appl. Environ. Microbiol. 61(6):2127-2131.