AGRICULTURE, FOOD & ANALYTICAL BACTERIOLOGY

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BRIEF COMMUNICATION
A Surveillance of Cantaloupe Genotypes for the Prevalence of *Listeria* and *Salmonella*

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ABSTRACT

Netting is a common characteristic in predominant cantaloupe (*Cucumis melo* L) varieties. Over the past several years, *Listeria* and *Salmonella* outbreaks associated with cantaloupes have become a subject of concern to consumers. It is hypothesized that unlike non-netted melons, the netted structure of the cantaloupe rind could be a host for pathogens. Therefore, we investigated whether pathogen contamination in the field setting is closely associated with the netted rind. Twenty one netted cantaloupe genotypes consisting of experimental F1 hybrids, inbred lines from the Texas A&M melon breeding program and commercial cultivars were tested for the presence of *Listeria* spp. and *Salmonella* serotypes. Pathogen isolation was performed using selective/differential media after pre-enrichment and selective enrichment. Use of selective media resulted in the occurrence of 36.36% false positives for *Listeria* spp. and 16.25% false positives for *Salmonella* serovars. Isolates were confirmed using biochemical tests (*Listeria* API and API 20E) for both pathogens and real time PCR for *Listeria*. Testing resulted in one of the triplicates in the cantaloupe breeding line, '1405' being positive for *Listeria innocua*. None of the genotypes were positive for *Salmonella* serovars indicating that there was a low prevalence of the pathogens in the melon genotypes tested in our study. The occurrence of false positives on selective/differential media highlights the importance of developing sound selective protocols for the detection and isolation of pathogens from cantaloupes. Understanding the natural prevalence of foodborne pathogens under growing conditions will help in developing field-based risk assessments for cantaloupes.

Keywords: cantaloupe lines, foodborne pathogens, field level assessment, rind netting, contamination, *Salmonella*. *Listeria*, false positives, PCR, chromogenic media

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INTRODUCTION

Fresh fruits and vegetables are profitable commodities for growers and distributors as consumption trends are on the rise (Hanning *et al*., 2009). Cantaloupes and honeydew melons are popular among the United States (US) consumers because of their sweetness and nutritional content (Ukuku and Sapers, 2007). The high antioxidant value of cantaloupes and the convenience of prepackaged ready to eat (RTE) fruits have further contributed to the popularity of this type of melon in the US (Lester and Hodges, 2008). However, sales of cantaloupes are often severely affected during and after an outbreak implication, making it important to mitigate contamination by pathogenic bacteria (Ribera *et al*., 2012).

Cantaloupes contaminated by foodborne pathogens such as *Listeria monocytogenes* and *Salmonella enterica* have resulted in widespread diseases and associated economic losses. In 2011, a multistate outbreak caused by *L. monocytogenes* contaminated cantaloupes resulted in 146 cases, 30 fatalities and one miscarriage (Laksanalamai *et al*., 2012). In 2012, another cantaloupe outbreak caused by *Salmonella* serotypes Typhimurium and Newport involving 24 states resulted in 261 cases, three deaths and 94 hospitalizations (CDC, 2012). These outbreaks highlight the susceptibility of cantaloupes to contamination by foodborne pathogenic bacteria. Various factors such as netting of the rind, presence of pathogens in soil, water and manure, hydrophobicity and attachment appendages of the pathogens, as well as biofilm formation could result in colonization of enteric pathogens on cantaloupe rinds (Ukuku and Sapers, 2007; Hanning *et al*., 2009). Rainfall, water runoff, underground water, and surface water currents can all aid in the dissemination of foodborne pathogens in soils and sediments (Bech *et al*., 2010).

Sweet melons such as the netted cantaloupe (*Cucumis melo* L. *reticulatus*) can get contaminated during pre-harvest operations in the field or during post-harvest processing (Ukuku and Sapers, 2007). Aggregates of foodborne pathogens on cantaloupe rinds could result in contamination of the fruit in the field and consequently cross-contamination of other fruits in packaging houses (Morris and Monier, 2003). The factors responsible for the 2011 *L. monocytogenes* contaminated cantaloupe outbreak were attributed to packing house design, ineffective equipment sanitation and lack of pre-cooling of melons before cold storage (Laksanalamai *et al*., 2012). Melons with a contaminated rind are a food safety risk, as pathogenic bacteria can potentially be transferred from the surface to the flesh by cutting tools (Lin and Wei, 1997), especially in RTE pre-cut products.

A study by Gagliardi *et al*. (2003) indicated that following post-harvest processing, there were higher bacterial counts on cantaloupe rind compared to those still in the field (Gagliardi *et al*., 2003). The inefficiency of washing is most likely due to the porous surface characteristics of cantaloupe and the increased roughness resulting from the microstructures present in the netting which could favor bacterial attachment (Webster and Craig, 1976; Chen *et al*., 2012). Averting on-field contamination of melons by pathogenic bacteria could be preemptive, as it is known that melon rinds retain bacteria even after washing and chemical sanitizer treatments (Sapers *et al*., 2001).

Using cantaloupe genotypes that have lower retention of foodborne pathogenic bacteria on their rinds could help reduce the risk of fruit contamination in the field. Determining genotypes of cantaloupes that are less susceptible to bacterial attachment and contamination could contribute to enhanced microbial safety of cantaloupes. Hence, the objective of this study was to survey the prevalence of *Listeria* and *Salmonella* spp. amongst various genotypes of cantaloupes harvested directly from the field.

MATERIALS AND METHODS

Cantaloupe genotypes

The test cantaloupes consisted of 14 experimental hybrids, three inbred lines and four commercial cultivars. These cantaloupe genotypes had been selected for high yield, disease resistance, and firm, high quality fruit (Crosby *et al*., 2006). Seeds of all genotypes were sown directly in a silty-clay soil at

the Texas A&M AgriLife Research Center, Uvalde, TX (long. 29°1'N, lat. 99°5'W, elevation 283 m) on March 30, 2012. Plants were grown with standard commercial practices of sub-surface drip irrigation and fertigation, on black plastic mulch at a spacing of 2 m between beds and 0.30 m between plants on the bed. All fruits were allowed to reach half slip maturity before harvesting. Slip is considered as the abscission zone between the fruit and peduncle. Half slip maturity is a standard commercial harvest procedure used by growers in Texas and other southern regions of the US. Net characteristics ranged from complete coverage, high off the epidermis (ropy) to sparse coverage of short netting. Some genotypes had a higher incidence of splitting in the net tracts than others. The majority had some resistance to *Fusarium* induced rind lesions, but some genotypes did exhibit this damage when fruit contacted the clay loam soil. Fruits were harvested between July 1 and July 7, 2012 and shipped to the Ravishankar laboratory in the Department of Veterinary Science and Microbiology (Currently School of Animal and Comparative Biomedical Sciences) at the University of Arizona within 2 days. No post-harvest methods were performed on the cantaloupes before analysis.

Storage and inspection

Upon arrival to the laboratory, cantaloupes were initially inspected for any visible damage or spoilage. The longitudinal circumference from the stem scar of each fruit was measured using a measuring tape. Cantaloupe fruits were given alternate numerical codes to prevent bias and maintain anonymity. Cantaloupes were stored at 4°C for 24 h and were evaluated for the presence of *Listeria* and *Salmonella*.

Surveillance of cantaloupes for *Listeria* spp.

Plugs of cantaloupe (20 mm length) were obtained from the stem scar, the bottom of the fruit and the sides of each fruit using a sterile cork borer (20 mm diameter) in order to collect both rind and flesh tissues. A total of 25 g of tissue was taken from each cantaloupe for sampling. Isolation of *Listeria* spp. was performed based on the procedure adapted from the "FDA-Bacteriological Analytical Manual (BAM) for the isolation of *L. monocytogenes* from foods" (Hitchins and Jinneman, 2011). Briefly, tissue samples were mixed with 225 ml of basal Buffered *Listeria* Enrichment Broth (BLEB) (EMD Chemicals Inc, Gibbstown, NJ) in a stomacher (Stomacher Lab-Blender 400, Tekmar Co., Cincinnati, OH) for 2 min and incubated for 4 h at 30°C. Following this, cycloheximide (Sigma-Aldrich, St. Louis, MO) was added and the suspension was incubated at 30°C for 48 h. After incubation, loopful of the suspensions were streaked on to petri dishes containing modified Oxford formulation (MOX; Becton, Dickinson and Co, Sparks, MD) agar and *Listeria* CHROMagar™ (CHROMagar, Paris, France) which were incubated at 37°C for 48 h. Typical black colonies formed on MOX and blue colonies on CHROMagar irrespective of halo formation were Gram stained and streaked for isolation to account for *Listeria* spp. Those colonies that were Gram positive were further confirmed using *Listeria* API strips (bioMerieux, Hazelwood, MO), accessory tests (catalase, oxidase and hemolysis) according to the manufacturer's instructions, and real time PCR (iQ Check *Listeria* spp. Kit, Bio-Rad laboratories, Hercules, CA)

Real time PCR confirmation of *Listeria* spp.

One isolated colony from a MOX plate was added to 100 µl of lysis buffer (Bio-Rad Laboratories) and incubated for 15 min at 95°C. To 45 µL of the PCR amplification master mix, 5 µL of the lysed DNA sample was added along with 5 µL fluorogenic oligonucleotide molecular beacon probe solution (iQ Check *Listeria* spp. Kit, Bio-Rad Laboratories). The thermocycler (MiniOpticon™ real-time PCR detection system, Bio-Rad Laboratories) was programmed as follows: 50°C for 2 min, 95°C for 5 min followed by 95°C for 20 s, 55°C for 30 s, 72°C for 30 s for 50 cycles, and 72°C for 5 min. An increase in fluorescence from the amplification of the target sequence resulting in a Ct value ≥10 was considered positive.

Surveillance of cantaloupes for *Salmonella* serovars

A total of 25 g of tissue was taken from each cantaloupe using techniques similar to those described earlier for *Listeria*. Isolation of *Salmonella* spp. was performed based on the procedure adapted from the "FDA-BAM for the isolation of *Salmonella* spp. from foods" (Andrews and Hammack, 2007). Briefly, tissue samples were mixed with 225 ml sterile universal pre-enrichment broth (UPB; Becton, Dickinson and Co.) for 2 min using a stomacher (Stomacher Lab-Blender 400, Tekmar Co.). The suspension was incubated for 24 h at 37°C following which 100 µl was transferred to 10 ml Rappaport-Vassiliadis (RV; EMD Chemicals Inc.) medium and another 1 ml to 10 ml tetrathionate (TT; EMD Chemicals Inc.) broth. The RV suspension was vortexed and incubated at 42°C for 24 h in a water bath. The TT broth suspension was vortexed and incubated at 37°C for 24 h. Following incubation, the suspensions from the broths were streaked on to xylose lysine desoxycholate (XLD) agar (Becton, Dickinson and Co.) and CHROMagar™ *Salmonella* (CHROMagar) and incubated for 48 h. Typical colonies were Gram stained and Gram negative isolates were confirmed as *Salmonella* using API 20E strips (bioMerieux), and by conducting biochemical tests (catalase, oxidase) according to the manufacturer's recommendations.

Statistical Analysis

Geometric means and standard deviations were calculated for the incidences of false positives on selective plating media. A t-test was performed to determine significant differences ($p<0.05$) between false positive rates on different selective media. Statistical analysis was performed using Microsoft Excel 2007 (Microsoft Corp., Seattle, WA). Means and standard deviations were calculated for the longitudinal circumference values of melons.

RESULTS

Sizes of cantaloupes based on their diameter

A total of 21 cantaloupe genotypes (3 fruits each for most genotypes) were surveyed for the presence of *Listeria* and *Salmonella* spp. The cantaloupe genotypes with the maximum average longitudinal circumference were lines 18 and 20 with 60.53±4.06 and 62.23±4.58 cm, respectively (Table 1). Cantaloupe breeding line 6 had the smallest melons with an average longitudinal circumference of 46.57±1.50 cm. Cantaloupe genotypes 17 and Oro Duro were also some of the smaller lines tested with an average longitudinal circumference of 50.37±2.61 cm and 49.97±1.44 cm, respectively.

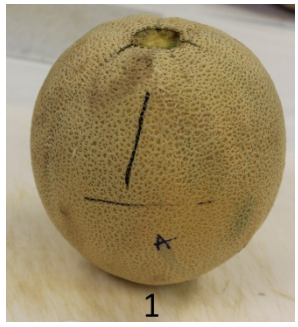
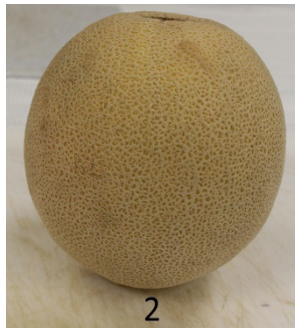
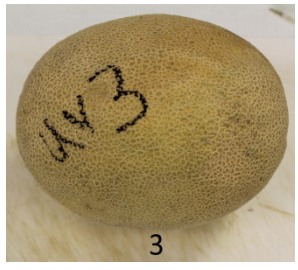
Surveillance of cantaloupes for *Listeria* spp.

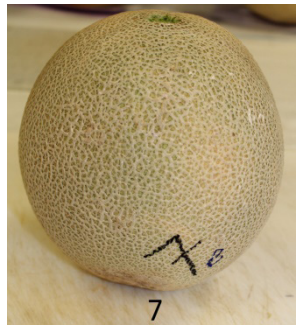
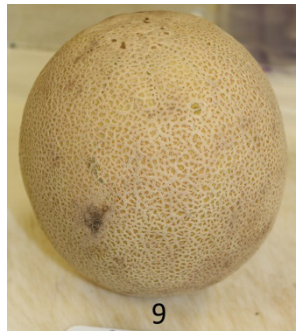
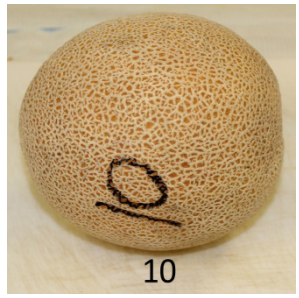
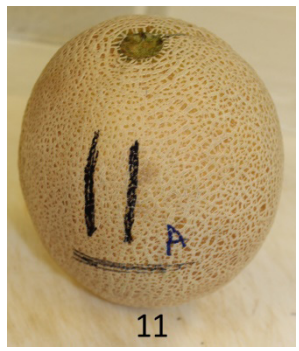
None of the 21 cantaloupe genotypes were positive for the presence of *L. monocytogenes*. One cantaloupe sample from the breeding line 1405 was positive for the presence of *L. innocua* after enrichment and plating on selective media (Table 1). This sample was further confirmed through *Listeria* API tests and real Time PCR. Real Time PCR analysis resulted in one isolate from cantaloupe line 1405 having an increase in the fluorescence curve indicating amplification and a Ct>10, indicating a positive result for *Listeria* spp. Sixteen of the 21 melon lines tested demonstrated black and blue colored colonies on MOX and *Listeria* CHROMagar, respectively. The blue colonies were chosen to determine the presence of other *Listeria* spp. All these colonies were Gram positive. However, the results of API *Listeria* test and real-time PCR indicated that 15 of these isolates were negative for *L. monocytogenes*.

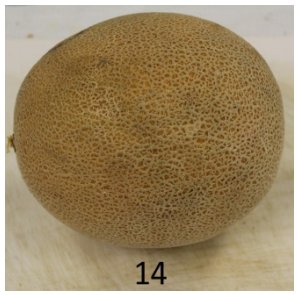
Surveillance of cantaloupes for *Salmonella*

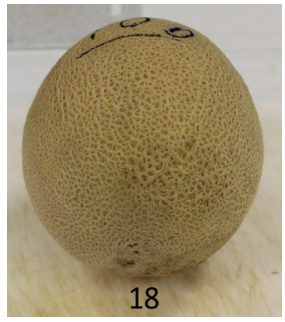
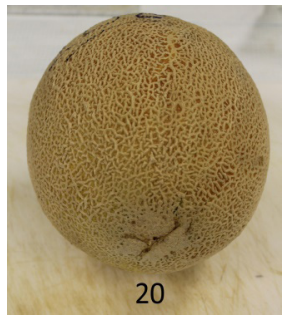
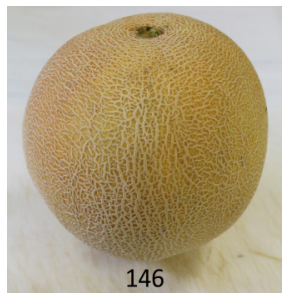
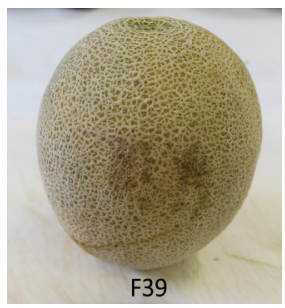
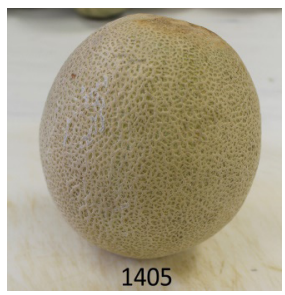
None of the 21 cantaloupe genotypes were positive for the presence of *Salmonella* serovars. Out of

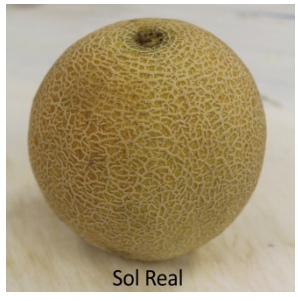
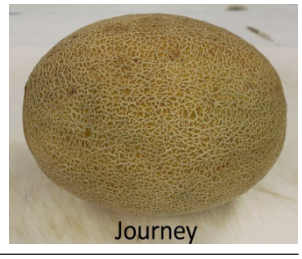
Table 1. Rind net characteristics and surveillance tests for the presence of *Listeria* spp. and *Salmonella* on various cantaloupe genotypes using API *Listeria* for *Listeria* and API 20E for *Salmonella*.

Cantaloupe genotype	Rind net characteristics			Pathogen surveillance test[z]		Photograph
	Longitudinal circumference (cm)	Net Coverage (%)	Splitting /corkiness	API *Listeria* strip	API 20E strip	
Experimental Hybrid						
1	50.37±2.62	100	Low	Neg	Neg	
2	52.90±0.69	100	Low	Neg	Neg	
3	54.60±1.30	100	Low	Neg	Neg	
6	46.57±1.50	90	Low	Neg	Neg	

Cantaloupe genotype	Rind net characteristics			Pathogen surveillance test[z]		Photograph
	Longitudinal circumference (cm)	Net Coverage (%)	Splitting /corkiness	API *Listeria* strip	API 20E strip	
7*	58.40	100	Low	Neg	Neg	
9	53.95±6.29	100	Low	Neg	Neg	
10	59.70±5.86	100	Low	Neg	Neg	
11	50.20±2.69	100	Low	Neg	Neg	

Cantaloupe genotype	Rind net characteristics			Pathogen surveillance test[z]		Photograph
	Longitudinal circumference (cm)	Net Coverage (%)	Splitting /corkiness	API *Listeria* strip	API 20E strip	
12*	53.30	100	Low	Neg	Neg	
14	54.17±1.50	100	Low	Neg	Neg	
15*	67.30	100	Low	Neg	Neg	
17	50.37±2.61	100	Low	Neg	Neg	

Cantaloupe genotype	Rind net characteristics			Pathogen surveillance test[z]		Photograph
	Longitudinal circumference (cm)	Net Coverage (%)	Splitting /corkiness	API *Listeria* strip	API 20E strip	
18	60.53±4.06	100	Med	Neg	Neg	
20	62.23±4.58	100	Med	Neg	Neg	
	Inbred					
146	51.67±3.87	100	Low	Neg	Neg	
F39	49.53±4.58	100	Low	Neg	Neg	
1405	57.60±1.93	100	Med	Pos+Neg	Neg	

Cantaloupe genotype	Rind net characteristics			Pathogen surveillance test[z]		Photograph
	Longitudinal circumference (cm)	Net Coverage (%)	Splitting /corkiness	API *Listeria* strip	API 20E strip	
Commercial variety						
Mission	53.33±2.55	100	Low	Neg	Neg	
Oro Duro	49.97±1.44	100	Low	Neg	Neg	
Sol Real	52.07±2.19	100	Low	Neg	Neg	
Journey	54.60±5.86	90	Med	Neg	Neg	

[z] Positive colonies isolated from selective media were tested for the presence of pathogen using an API *Listeria* strip for *Listeria* and an API 20E strip for *Salmonella*. Results were Negative (Neg) or Postive (Pos) for *Listeria* or *Salmonella*. One of the triplicates in 1405 showed positive for *Listeria* while two other replicates came out negative.

[*] Only one sample was available in experimental hybrid 7, 12 and 15, because it is difficult to synchronize the maturity of all genotypes and open pollinated fruits in a field trial or some fruits may have aborted during fruit development.

the 21 cantaloupe genotypes tested, 8 genotypes- 3, 9, 10, 18, 146, 1405, Mission and Oro Duro showed typical black colonies on XLD agar indicative of H_2S production and typical mauve colonies on *Salmonella* CHROMagar. All the isolates from the 8 lines were Gram negative rods. However, when API 20E tests were conducted, all 8 were negative for the presence of *Salmonella* serovars (Table 1). A total of 10 and 8 melons (from the 8 genotypes tested) resulted in false positives on XLD agar and *Salmonella* CHROMagar, respectively. The same melons from genotypes- 9, 18, 146, 1405, and Oro Duro resulted in false positives on both selective media, while different melons from genotypes 3, 10 and Mission resulted in false positives on XLD agar and *Salmonella* CHROMagar.

DISCUSSION

A total of 21 cantaloupe genotypes were surveyed for the presence of *Salmonella* and *Listeria* spp. Of all the lines surveyed, line 1405 was positive for the presence of *L. innocua* (Table 1). This is an inbred line with a typical, complete net of medium height and low incidence of splitting. The heavier net completely covers the rind (most Western shipper cantaloupes) and could result in improved attachment of microbiota.

L. innocua has been used as a surrogate for *L. monocytogenes* (Buchholz *et al*., 2011), because of similar growth and survival characteristics (McKinney *et al*., 2009). *L. monocytogenes* might be capable of surviving in similar or harsher environments than *L. innocua* (Buchholz *et al*., 2011). The presence of *L. innocua* on cantaloupe could indicate conditions suitable for the possible survival and contamination by *L. monocytogenes*.

L. monocytogenes is commonly found in the environment and on plant material (Laksanalamai *et al*., 2012) and can survive under adverse environmental conditions (Tompkin, 2002). Johnston *et al*., (2005) surveyed cantaloupes and other produce from the southern regions of the US for the presence of pathogens (*L. monocytogenes* and *Salmonella* serovars) and indicator organisms. Of the 398 produce items sampled, none were positive for *L. monocytogenes*. Of all the produce tested for *Salmonella*, three of the 90 cantaloupes were positive for *Salmonella* Montevideo (Johnston *et al*., 2005). While lower numbers of contaminated produce can occur in the field, cross-contamination in the packing house may result in higher volumes of product getting contaminated and thereby causing outbreaks.

In our study, the cantaloupe genotypes tested were not positive for *Salmonella*. The use of manure, or presence of wild life, birds, or compost piles in the field vicinity could serve as reservoirs of contamination. While the research farm at the Texas A&M AgriLife Research Center did not contain these pathogen reservoirs, farms could potentially be subjected to pathogen introduction through environmental contamination and animal or bird intrusion. Previous studies have indicated that foodborne pathogens can survive in soil and water for extended periods of time and can be transferred to fruit tissue (Baloda *et al*., 2001; Gupta *et al*., 2007; Barak and Liang, 2008). Factors that affect the survival of the pathogen in soil include soil type, nutrient availability, manure and temperature (Andrews-Polymenis *et al*., 2010).

Selective isolation of pathogens from cantaloupes resulted in false positive samples for both *Listeria* spp. and *Salmonella* on selective media. In a study to evaluate chromogenic agar media for the recovery and detection of *L. monocytogenes* in foods, it was observed that natural microbiota in foods are capable of overgrowing pathogenic target microorganisms (Michael, 2004). In our study, the sensitivity of non-chromogenic plating media was not significantly different ($P>0.05$) from chromogenic plating media for distinguishing false positives, in case of both pathogens.

Cantaloupes are rich in sugars and the rinds of cantaloupes are capable of harboring high amounts of microbiota because of the naturally present netting (Ukuku and Sapers, 2007). The breakage or rupture of the rinds could potentially result in cross-contamination of the microorganisms from one fruit to another, due to spilling of the juice, which is rich in antioxidants and sugars (Lester and Hodges, 2008).

While our study indicated that the various genotypes did not result in significant differences in pathogen attachment, mitigation strategies should be explored to reduce the risk of on-field contamination of cantaloupes.

CONCLUSIONS

A survey of 21 cantaloupe genotypes resulted in a single line of netted cantaloupe being positive for *Listeria* spp., which was confirmed as *L. innocua*. The presence of *L. innocua* on cantaloupe indicates the existence of conditions wherein pathogenic *L. monocytogenes* could survive. More research is needed to understand the role of cantaloupe netting on microbial attachment and persistence.

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