Final Report to American Meat Institute Foundation

Project Title:

The role of aerosols in transmission of microorganisms (including *Listeria*) to ready-to-eat meat/poultry products

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Project Summary

Airborne contamination of *Listeria monocytogenes* in food processing facilities may or may not be an important contributing factor in disseminating *L. monocytogenes* in such facilities. However, aerosol studies in food processing plants have been limited by lack of a suitable surrogate microorganism for *L. monocytogenes*. The objectives of this study were to investigate the potential of using *Jonesia denitrificans* as a surrogate for aerosol studies of *L. monocytogenes* and to study the role of aerosol in transmission of microorganisms (including *L. monocytogenes*) to ready-to-eat meat/poultry products in a bioaerosol containment chamber as well as in a small-scale poultry deboning room (*J. denitrificans* only).

The settling rates of aerosol-borne *L. monocytogenes* and *J. denitrificans* released into a bioaerosol containment chamber were monitored. Results revealed that settling rates depend on particle size and relative humidity of the environment. Larger particles settled from the air more rapidly than smaller particles, with 5-um particles largely removed from the air within a few minutes of release and 0.3-um particles remaining airborne (<1 log reduction) for 3 to 4 hours. In most instances, relative humidity (RH) at 40 or 75% had a minimal effect on settling rates, although settling rates of both organisms were slightly greater at 75% RH than at 40% RH. Overall, *J. denitrificans* had similar settling rates as *L. monocytogenes*.

The contamination level of *J. denitrificans* on turkey meat following its aerosolization in a bioaerosol containment chamber was similar to that of *L. monocytogenes* which was largely dependent on initial cell numbers and exposure time. The greater the number of cells in the aerosol, the greater the number of contaminated turkey samples and the less exposure time for contamination to occur. No turkey samples were *J. denitrificans*- or *L. monocytogenes*-positive within 4 hours of exposure when the initial cell numbers were $\leq 2.5 \times 10^2$ or $\leq 1.5 \times 10^2$ cfu/L air, and all samples were positive within 5 to 30 minutes of exposure when initial cell numbers were $\geq 3.5 \times 10^5$ cfu/L air. More samples were positive in the 75% RH environment than in 40% RH when the inoculum was 10^3 cfu/L air, but relative humidity had little influence on the number of contaminated samples for higher or lower levels of inoculum. The detectable cell

numbers of *J. denitrificans* and *L. monocytogenes* on positive samples of non-cured turkey meat were generally low, ranging from 1 to 12 cfu per three slices. This suggests that even when relatively large cell numbers are initially present in aerosols in a room, relatively small cell numbers of *L. monocytogenes* surface contaminate product during a short exposure time.

Studies on the survival and growth of *J. denitrificans* and *L. monocytogenes* on non-cured turkey meat with no preservatives at 4 and 12ºC with and without vacuum packaging revealed that *J. denitrificans* does not grow at 4ºC and cell numbers decrease during 7 days at 12ºC, whereas *L. monocytogenes* grows well at both 4 and 12ºC within 4 to 7 days independent of type of packaging. Hence, *J. denitrificans* is not a suitable surrogate for *L. monocytogenes* for persistence and growth studies.

Releasing *J. denitrificans* at 10^3 cfu/L as an aerosol into a deboning room of a small-scale poultry processing facility revealed that the distance from the air conditioning units from which the bacteria were aerosolized influenced the amount of *J. denitrificans* contamination that occurred. The greatest degree of contamination occurred at 100 to 150 cm from the air conditioners, and least at 50 and 250 cm from the units. For samples obtained at 100 cm, the greatest average number of *J. denitrificans* on agar media was $2.4x10^2$ cfu/plate, and greatest percentage of meat samples positive at a sampling distance was 40%. Results indicate that when aerosolized at a high population (10^3 cfu/L), *J. denitrificans* can contaminate agar plate and meat surfaces at a range of 250 cm from air conditioning units, with the greatest degree of contamination occurring within 100 to 150 cm of an air conditioning unit. Interestingly, swab sampling of environmental surfaces of the deboning room immediately after aerosolizing *J. denitrificans* yielded negative results; indicating *J. denitrificans* is not a good environmental survivor.

Phase I
Objectives:
1) Determine settling rates of aerosol particle size and *L. monocytogenes* in a bioaerosol containment chamber
2) Determine cross-contamination of aerosolized *L. monocytogenes* on turkey meat in a bioaerosol containment chamber

Materials and Methods

Bacterial strains. A five-strain mixture of *L. monocytogenes* (101M, L 498, L. BM, L34, and L81) was used. Each strain was cultured individually in 10 ml of tryptic soy broth with 0.6% yeast extract (TSB-YE) for three successive 24-h intervals at 37°C. After incubation, each culture was sedimented twice by centrifugation at 5000 x *g* for 10 min, then washed twice and resuspended in Butterfield's phosphate buffer (BPB, pH 7.2). For each pathogen, equal portions of each bacterial strain were combined and used as the inoculum. Optical density at 600 nm of the combined bacterial suspension was adjusted at 1.0 (ca. 10^9 cfu/ml). The bacterial count of the 5-strain mixture was determined by plating 0.1-ml portions of appropriate dilutions onto duplicate plates of tryptic soy agar (TSA)-modified Oxford (MOX) overlaid plates, following incubation at 37°C for 24 h.

Aerosolization of *L. monocytogenes* in a bioaerosol containment chamber. A stainless steel tray with a rack on the top was placed on the bottom of the containment chamber. Saturated salt solution was placed in the tray to maintain a constant relative humidity within the chamber. Seventy-five or 40% relative humidity was maintained by saturated NaCl or $MgCl_2$, respectively, in the chamber. The interior temperature of the chamber was maintained at 22 to 23°C throughout the study. The containment chamber was comprised of plexiglass with dimensions of 124 cm (length) x 51 cm (width) x 51 cm (height) (315 L) (see Attachment A). The chamber was constructed with a 5- µm air

filter and openings for air sampler hose connections, and two gloves hermetically sealed to two ports. Two hoses on each side of the chamber were connected to an air pump. The Petri dishes with deli meats were sealed with Parafilm and placed evenly on a rack. Bacterial suspension (8 ml) was placed into a nebulizer, which was placed on top of a test tube rack in the middle of the chamber. Release time of the nebulizer was adjusted to 10 min. The air pump was turned on while releasing *L. monocytogenes* to increase airflow. When the nebulizer stopped, the two hoses were switched to different connections of the air pump to change the airflow in the opposite direction for 10 min. Immediately after air circulation was stopped, the Parafilm on the Petri dishes was removed, and then lids of the Petri dishes were removed to expose the deli meats. Three Petri dishes at different locations in the chamber were covered after 5, 30, 60, 120, and 180 min of exposure. At low humidity (40%), three extra slices of meats were exposed for 240 min. Two unopened Petri dishes sealed with Parafilm served as negative controls and two unopened Petri dishes not sealed with Parafilm served as another set of controls.

Settling rates of *L. monocytogenes*. *L. monocytogenes* was released into the bioaerosol containment chamber at 10^7cfu/L air. Ten MOX plates overlaid with tryptic soy agar containing 0.6% yeast extract (TSA-YE) were placed upside-down on the bottom of the chamber while *L. monocytogenes* was being released. Following aerosolization, plates were inverted and exposed for 30, 60, 90, 120, 150, and 180 min. Two time intervals using five plates for each were tested for each trial. Average *L. monocytogenes* counts from the five TSAYE/MOX plates over time were used to determine settling rates for *L. monocytogenes*. Duplicate studies were done at each humidity.

Particle size and suspension time of the aerosolized suspension of *L. monocytogenes*. *L. monocytogenes* was released into the bioaerosol containment chamber at 10^7 cfu/L air. Numbers of *L. monocytogenes* particle sizes ranging from 0.3, 0.5, 1, 2, and 5 µm were measured by a particle monitor (Met One Instrument, Model GT-321, Grants Pass, OR) at 0, 30, 60, 90, 120, 150, and 180 min. Baseline data of particle numbers in the air prior to aerosolization of *L. monocytogenes* was determined before the listeriae bioaerosol was released into the chamber. Hence, the numbers of particle sizes delivered by aerosolization of *L. monocytogenes* were determined as the difference between initial counts (baseline) and the count at each exposure time. Duplicate studies were done at both humidities.

Ready-to-eat meat products. Two types of deli meats, cured turkey ham containing sodium nitrite and potassium lactate and non-cured oven-roasted turkey, were tested. Presliced-packaged deli meats were purchased in a local store and stored at 4°C. Total moisture, fat, and salt content were determined by AOAC methods and pH values were obtained by a flat-surface pH probe. On the day of the experiment, a slice of deli meat was placed in a sterile 100 x 15-mm Petri dish, cut by a sterile 60 x 15-mm Petri dish, and then placed into another sterile 60 x 15-mm Petri dish.

Exposure of ready-to-eat meat products in the bioaerosol containment chamber. Non-cured turkey meat containing no antimicrobials to *L. monocytogenes* was used. Petri dishes with turkey meat were sealed with Parafilm and placed evenly on a rack. Immediately after the air circulation was stopped, the Parafilm on the Petri dishes was removed and the turkey meats were exposed. Three Petri dishes at different locations in the chamber were covered with lids at 5, 30, 60, 120, and 180 min of exposure. At 40%

relative humidity, three extra Petri dishes of meat were exposed for 240 min. Two unopened Petri dishes sealed with Parafilm served as negative controls, and two unopened Petri dishes with no Parafilm also served as controls. Four inoculation levels, 10^1, 10^2, 10^3, and 10^5 cfu/L air, were tested at both relative humidities.

Detection and enumeration of *L. monocytogenes*. Detection and identification procedures were based on the USDA Microbiology Laboratory Guidebook, 3rd ed. (1998). *L. monocytogenes* counts on turkey meat were determined by direct plating and only conducted on non-cured turkey meats. Deli meats in the Petri dishes were added to Stomacher bags with 75 ml (5x vol/wt.) of University of Vermont broth (UVM). A slice of meat, which was not placed into the chamber, was added to a Stomacher bag, and inoculated with 3 to 5 cfu of the five-strain mixture of *L. monocytogenes* to serve as a positive control. After maceration by stomaching at 230 rpm for 2 min, 0.25 or 0.1 ml of the homogenate was spread onto four or two TSA-MOX overlaid plates, respectively. The remaining homogenate was incubated at 30°C for 24 h. After incubation, 0.1 ml of meat homogenate was transferred to Fraser broth and a loopful of the homogenate was streaked onto MOX plates. Both MOX plates and Fraser broth were incubated at 37°C for 24 h. Presumptive *L. monocytogenes* -positive cultures in Fraser broth were streaked onto MOX plates, and incubated at 37°C for 24 h. Presumptive *L. monocytogenes*-positive colonies on MOX plates were streaked onto horse blood (HB) overlay agar plates, and then incubated at 37°C for 24 h. Well-isolated colonies exhibiting hemolysis on HB plates were streaked onto TSA-YE plates and into BHI broth. Both TSA-YE plates and BHI broth were incubated at 25°C for 24 h. Colonies showing Henry illumination (bluish around colonies) on TSA-YE plates were confirmed by biochemical

assays (API Listeria; bioMériux Inc., Hazelwood, MO) and catalase test. BHI suspension was examined microscopically for cell morphology, tumbling activity, and gram stain. Upon completion of the experiment, remaining *L. monocytogenes* cell suspension in the nebulizer was enumerated by plating serial dilutions in BPB on TSA-MOX plates. *L. monocytogenes* cell numbers released in the containment chamber were determined by calculating the difference between the initial and remaining populations in the nebulizer.

Results

Particle size and settling rates of the aerosolized suspension *L. monocytogenes.* Large aerosolized particles sedimented more rapidly than small particles, and suspension time was slightly longer in a high humidity (75%) than in a low (38%) humidity environment (Fig. 1 and 2). Settling rates of *L. monocytogenes* were similar at both 38% and 75% RH (Fig. 3).

Proximate analyses of deli meats. Results of proximate analyses of the two types of turkey deli meat were similar. Total moisture, fat and salt contents were 73.5, 5.2, and 1.4%, respectively. The pH value ranged from 6.2 to 6.3. Variations between the types and batches of turkey meats for moisture, fat, and salt content were within 1%.

Cross-contamination of turkey meat by aerosolized *L. monocytogenes* in a bioaerosol containment chamber. The number of *L. monocytogenes* (Lm)-positive samples correlated with the initial number of cells aerosolized. All meat samples were Lm-positive when the highest initial cell numbers, 10^5 cfu/L air, were aerosolized. More Lm-positive samples were detected in the high than the low humidity environment when

ca.10^3 cfu/L were aerosolized. There was no significant difference between Lm-positive cured and non-cured turkey meat samples (Tables 1 and 2). *L. monocytogenes* cell numbers on most positive non-cured meat samples were too low to be recovered by direct plating, especially when low initial cell numbers were aerosolized (Table 3).

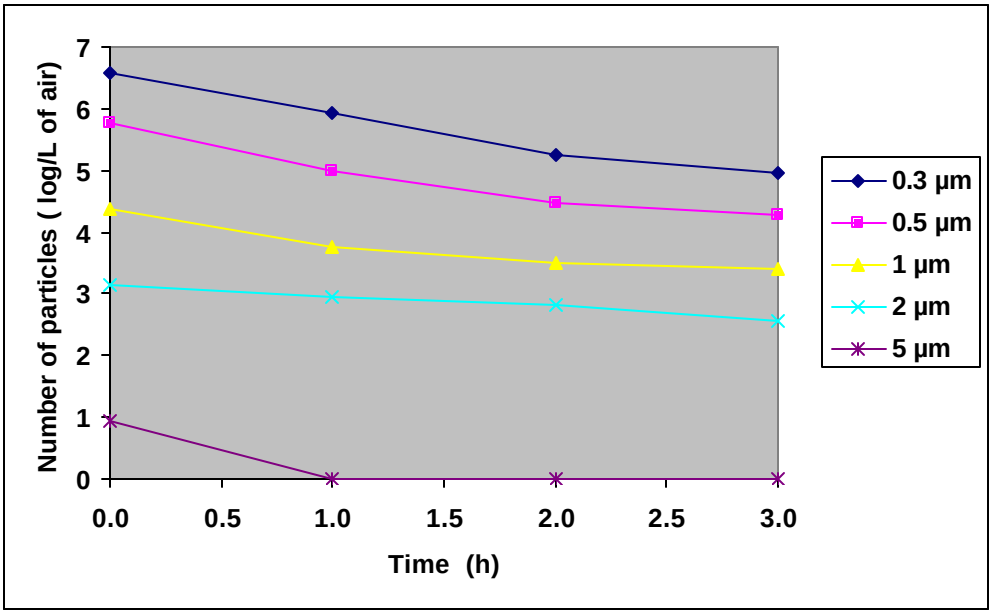

Fig. 1. Dynamics of sedimentation of particles in relation to their size when *L. monocytogenes* was aerosolized in a bioaerosol containment chamber at 38% relative humidity and room temperature (22-23^{o}C)

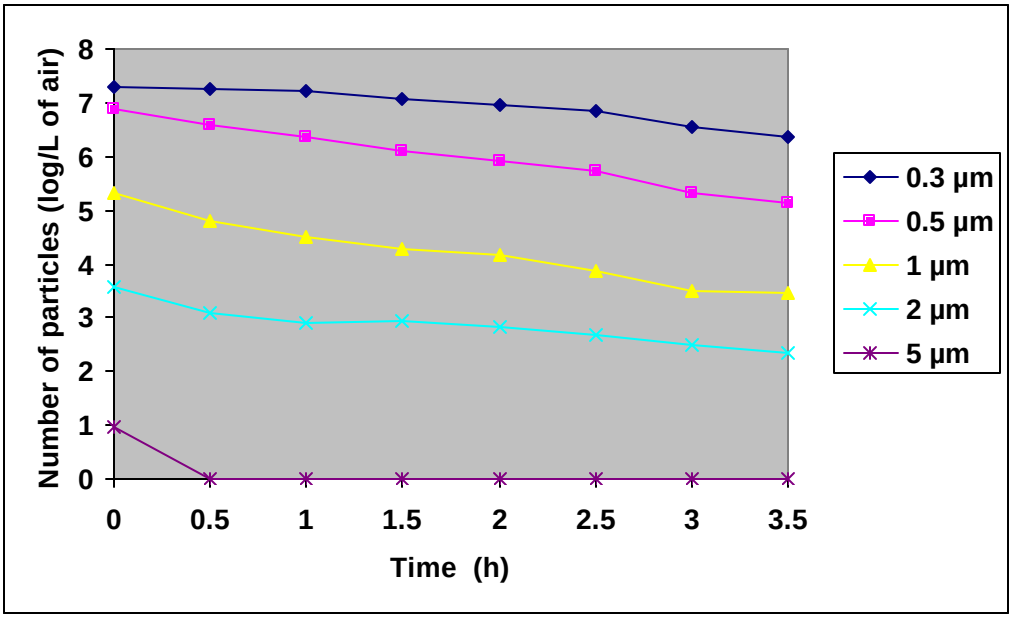

Fig. 2. Dynamics of sedimentation of particles in relation to their size when *L. monocytogenes* was aerosolized in a bioaerosol containment chamber at 75% relative humidity and room temperature (22-23 ^{o}C)

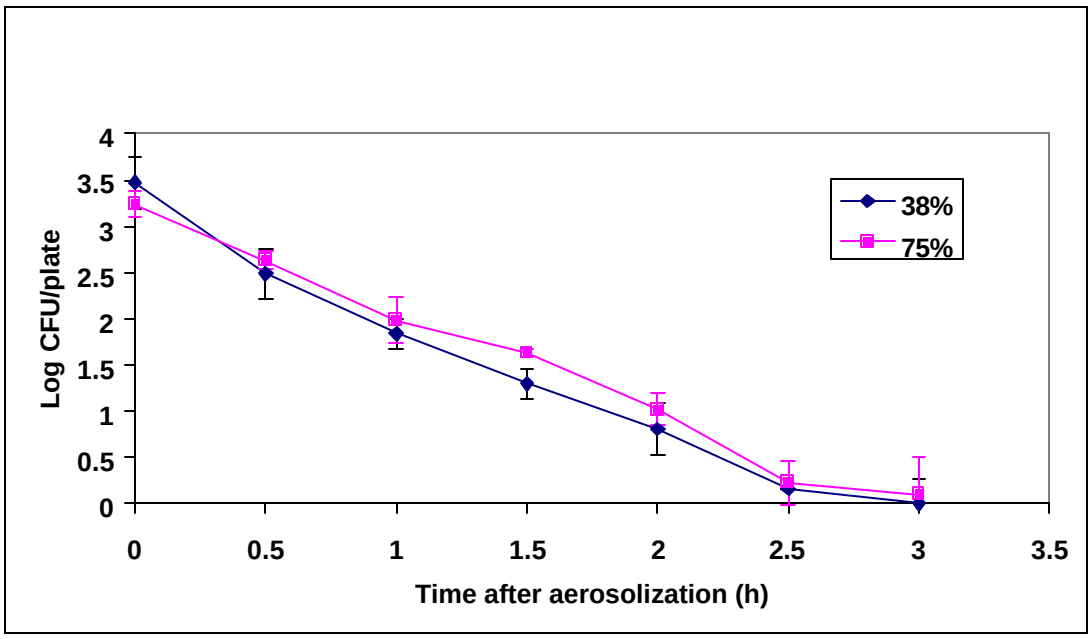

Fig. 3. Comparison of settling rates of *L. monocytogenes* in a bioaerosol containment chamber at 38 and 75% relative humidity and room temperature (22-23 ^{o}C)

Table 1. Number of *L. monocytogenes*-positive samples on cured turkey ham following aerosolization at room temperature (22-23°C) of listeriae at 40 and 75% relative humidity (RH) for different exposure times.

40% RH

Initial cell number[a]	Exposure time (min)						
(cfu/L air)	Control	5	30	60	90	180	240
44	0/3[b]	0/3	0/3	0/3	0/3	0/3	0/3
2.2×10^3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
1.0×10^5	0/3	2/3	3/3	3/3	3/3	3/3	3/3
6.6×10^5	0/3	3/3	3/3	3/3	3/3	3/3	3/3

75% RH

Initial cell number[a]	Exposure time (min)					
(cfu/L air)	Control	5	30	60	90	180
33	0/3	0/3	0/3	0/3	0/3	0/3
1.6×10^2	0/3	0/3	0/3	0/3	0/3	1/3
7.1×10^3	0/3	1/3	2/3	0/3	1/3	3/3
7.0×10^5	0/3	3/3	3/3	3/3	3/3	3/3

[a]Initial cell number of *L. monocytogenes* introduced into air by a nebulizer.

[b]Number of *L. monocytogenes*-positive samples/total number of meat samples at each exposure time. *L. monocytogenes* was detected by culture enrichment; *L. monocytogenes* cell numbers were not determined.

Table 2. Number of *L. monocytogenes*-positive samples on non-cured turkey meat following aerosolization at room temperature (22-23°C) of listeriae at 40 and 75% relative humidity (RH) for different exposure times.

40% RH

Initial cell number[a]	Exposure time (min)						
(cfu/L air)	Control	5	30	60	90	180	240
58	0/3 [b]	0/3	0/3	0/3	0/3	0/3	0/3
$1.9x10^2$	0/3	0/3	0/3	0/3	0/3	0/3	0/3
$1.3x10^3$	0/3	0/3	0/3	0/3	0/3	0/3	1/3
$3.5x10^5$	0/3	2/3	3/3	3/3	3/3	3/3	3/3

75% RH

Initial cell number[a]	Exposure time (min)					
(CFU/L air)	Control	5	30	60	90	180
27	0/3	0/3	0/3	0/3	0/3	0/3
$1.5x10^2$	0/3	1/3	0/3	0/3	0/3	0/3
$1.0x10^3$	0/3	1/3	2/3	3/3	3/3	3/3
$4.5x10^5$	0/3	3/3	3/3	3/3	3/3	3/3

[a]Initial cell number of *L. monocytogenes* introduced into air by a nebulizer.

[b]Number of *L. monocytogenes*-positive samples/total number of meat samples at each exposure time. *L. monocytogenes* was detected by culture enrichment.

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Table 3. *L. monocytogenes* cell numbers (cfu/three slices) on non-cured turkey meat following aerosolization at room temperature (22-23°C) of listeriae at 40 and 75% relative humidity (RH) for different exposure times.

40% RH

Initial cell number[a]	Exposure time (min)						
(cfu/L air)	Control	5	30	60	90	180	240
58	0	0	0	0	0	0	0
$1.9x10^2$	0	0	0	0	0	0	0
$1.3x10^3$	0	0	0	0	0	0	0
$3.5x10^5$	0	1	1	9	2	2	4

75% RH

Initial cell number[a]	Exposure time (min)					
(cfu/L air)	Control	5	30	60	90	180
27	0	0	0	0	0	0
$1.5x10^2$	0	0	0	0	0	0
$1.0x10^3$	0	1	0	1	2	2
$4.5x10^5$	0	2	6	6	8	11

[a]Initial cell number of *L. monocytogenes* introduced into air by a nebulizer.

Phase II

Objectives:

1) Determine settling rates of aerosol particle size and *J. denitrificans* in a bioaerosol containment chamber

2) Determine cross-contamination of aerosolized *J. denitrificans* on turkey meat in a bioaerosol containment chamber

3) Determine survival and growth of *J. denitrificans* and *L. monocytogenes* on non-cured turkey meat

Materials and Methods

Bacterial strains. *Jonesia denitrificans* (formerly: *Listeria denitrificans*) was used as a surrogate for *L. monocytogenes*. *J. denitrificans* was cultured in 10 ml of tryptic soy broth with 0.6% yeast extract (TSB-YE) for three successive 24-h intervals at 37°C. After the third transfer, the bacterial culture was washed twice following sedimentation by centrifugation at 5000 x *g* for 10 min, then resuspended in Butterfield's phosphate buffer, pH 7.2 (BPB). Optical density at 600 nm of the *J. denitrificans* suspension was adjusted to 1.0 (ca. 10^9 cfu/ml) and cell numbers were determined by plating 0.1-ml portions of appropriate dilutions onto duplicate plates of tryptic soy agar (TSA), following incubation at 37°C for 24 h.

Release of *J. denitrificans* in a bioaerosol containment chamber. The same bioaerosol containment chamber used to study the aerosolization of *L. monocytogenes* was used for *J. denitrificans* aerosolization studies. A stainless steel tray with a rack on the top was placed on the bottom of the chamber. A saturated salt solution was placed in the tray to maintain a constant relative humidity within the chamber. Seventy-five or 40% relative humidity was maintained by saturated NaCl or $MgCl_2$ solutions, respectively, in the chamber. The interior temperature of the chamber was maintained at 22 to 23°C throughout the study. Four inoculation levels, 10^1, 10^2, 10^3, and 10^5 cfu/L air, were tested at both relative humidities. Bacterial suspension (8 ml) was placed into a

nebulizer, which was placed on top of a test tube rack in the middle of the chamber. Release time of the nebulizer was adjusted to 10 min. The air pump was turned on while releasing *J. denitrificans* to increase airflow. When the nebulizer stopped, the two hoses were switched to different connections of the air pump to change the airflow in the opposite direction for 10 min.

Detection of *J. denitrificans*. Although *J. denitrificans* is closely related to *L. monocytogenes*, *J. denitrificans* does not grow in selective media commonly used for culturing *L. monocytogenes*. Hence, a modified enrichment broth and selective agar plates were used to detect and enumerate *J. denitrificans*. The enrichment broth, *Jonesia* enrichment broth (JEB), contained the same ingredients as UVM broth but without nalidixic acid. In addition, Polymyxin B (1000 units/L) and ferric ammonium citrate (0.005 g/L) were added to JEB. The modified selective agar was comprised of an equal amount of Columbia blood agar base and modified Oxford agar (CBA-MOX). *J. denitrificans* hydrolyzes esculin and forms black colonies on CBA-MOX. *J. denitrificans* is non-hemolytic, catalase-positive, and Voges-Proskauer test (VP)-negative. Consistent biochemical identification numbers were obtained for *J. denitrificans* with API Listeria test strips (bioMériux Inc., Hazelwood, MO). Hence, API-Listeria strips were used to confirm colonies of *J. denitrificans* even though the organism is not on the API list of identifiable species.

Settling rates of *J. denitrificans*. *J. denitrificans* was released into the bioaerosol containment chamber at 10^7 cfu/L air. Ten CBA-MOX plates overlaid with tryptic soy agar containing 0.6% yeast extract (TSA-YE) were placed upside-down on the bottom of the chamber while *J. denitrificans* was being released. Following aerosolization, plates

were inverted and exposed for 30, 60, 90, 120, 150,180, or 240 min. Two time intervals using five plates for each were tested for each trial. Average *J. denitrificans* counts from the five TSAYE/CBA-MOX plates over time were used to determine settling rates for *J. denitrificans*. Duplicate studies were done at each humidity.

Particle size and suspension time of aerosolized *J. denitrificans.* *J. denitrificans* was released into the bioaerosol containment chamber at 10^7 cfu/L air. Numbers of *J. denitrificans* particle sizes ranging from 0.3, 0.5, 1, 2, and 5 µm were measured by a particle monitor (Met One Instrument, Model GT-321, Grants pass, OR) at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min. Baseline data of particle size number within the chamber was determined before aerosolization. Hence, the numbers of the different particle size delivered by aerosolization of *J. denitrificans* were calculated as the difference of the count at each exposure time and initial counts (baseline). Duplicate studies were done at each humidity.

Exposure of ready-to-eat meat products in the bioaerosol containment chamber. Non-cured turkey meat containing no antimicrobials to *L. monocytogenes* was used. Petri dishes with turkey meat were sealed with Parafilm and placed evenly on a rack. Immediately after the air circulation was stopped, the Parafilm on the Petri dishes was removed and the turkey meats were exposed. Three Petri dishes at different locations in the chamber were covered with lids at 5, 30, 60, 120, and 180 min of exposure. At 40% relative humidity, three additional Petri dishes with meat were exposed for 240 min. Two unopened Petri dishes sealed with Parafilm served as negative controls, and two unopened Petri dishes with no Parafilm also served as controls.

Detection and enumeration of *J. denitrificans*. Detection and identification procedures for *L. monocytogenes* described in the USDA Microbiology Laboratory Guidebook, 3rd ed. (1998) were modified for detecting and enumerating *J. denitrificans*. Meat from a Petri dish was transferred to a Stomacher bag with 75 ml (5x vol/wt) of JEB. A slice of meat, which was not placed in the bioaerosol containment chamber, was transferred to a Stomacher bag and inoculated with 3 to 5 cfu of *J. denitrificans* to serve as a positive control. After maceration by stomaching at 230 rpm for 2 min, 0.25 or 0.1 ml of the homogenate was spread onto four or two CBA-MOX plates, respectively. The remaining homogenate was incubated at 30°C for 24 h for enrichment culture. After incubation, a loopful of the homogenate was streaked onto CBA-MOX plates and incubated at 37°C for 24 to 48 h. Presumptive *J. denitrificans*-positive colonies on CBA-MOX plates were tested for catalase activity. Catalase-positive colonies were streaked onto horse blood (HB) overlay agar plates and incubated at 37°C for 24 h. Well-isolated colonies without hemolysis on HB plates were confirmed by API biochemical assay and the VP test. Following aerosolization, the remaining bacterial suspension in the nebulizer was enumerated for *J. denitrificans* by plating serial dilutions (1:10) onto duplicate CBA-MOX plates. *J. denitrificans* cell numbers released in the chamber were determined as the difference between initial counts and the counts of the cell suspension remaining in the nebulizer.

Survival and growth of *J. denitrificans* and *L. monocytogenes* on non-cured turkey meat. Five slices (about 120 g) of non-cured turkey were placed into a sterile plastic package and the surface of the meat was inoculated with *J. denitrificans* or *L. monocytogenes* 101M at 10^2 cfu/package. Half of the packages was vacuum-sealed and

the other half was not vacuum-sealed, and were then held at 4 or 12°C. *J. denitrificans* or *L. monocytogenes* counts were determined at 4 and 7 days.

Results

Particle size and settling rates of the aerosolized suspension of *J. denitrificans*. The aerosolized suspension of *J. denitrificans* had similar particle size counts, suspension time and settling rates as *L. monocytogenes*. Large aerosolized particles sedimented more rapidly than small particles, and suspension time was longer in high humidity (75% RH) than in a low humidity (40% RH) environment (Fig. 4 and 5). Settling rates of *J. denitrificans* were slightly greater at 75% RH than at 40% RH (Fig. 6).

Cross-contamination of turkey meat by aerosolized *J. denitrificans* in a bioaerosol containment chamber. The number of *J. denitrificans* (JD)-positive samples correlated with the initial number of cells aerosolized in the chamber. All meat samples were JD-positive when the highest initial cell number, 10^5 cfu/L air, was in the air. More JD-positive samples were in the 75% RH environment than at 40% RH (Table 4). However, *J. denitrificans* counts could not be obtained for some of the JD- positive meat samples likely because of the low cell numbers. *J. denitrificans* counts (total number on three slices) on positive meat samples at each exposure time are shown in Table 5.

Survival and growth of *J. denitrificans* and *L. monocytogenes* on non-cured turkey meat. *J. denitrificans* was not detected on inoculated turkey meat after 4 or 7 days of storage at 4°C and a 1-log reduction of *J. denitrificans* occurred after 7 days at 12°C, regardless of whether meat was vacuum or not vacuum packaged (Table 6). *L. monocytogenes* grew prolifically within 7 days at either 4 or 12 °C. Results indicate that

J. denitrificans does not survive well at 4 °C and did not grow on turkey meat at 12 °C, whereas *L. monocytogenes* grew at both temperatures. Hence, *J. denitrificans* is not a suitable surrogate for *L. monocytogenes* for studying persistence or growth in the environment or on food surfaces.

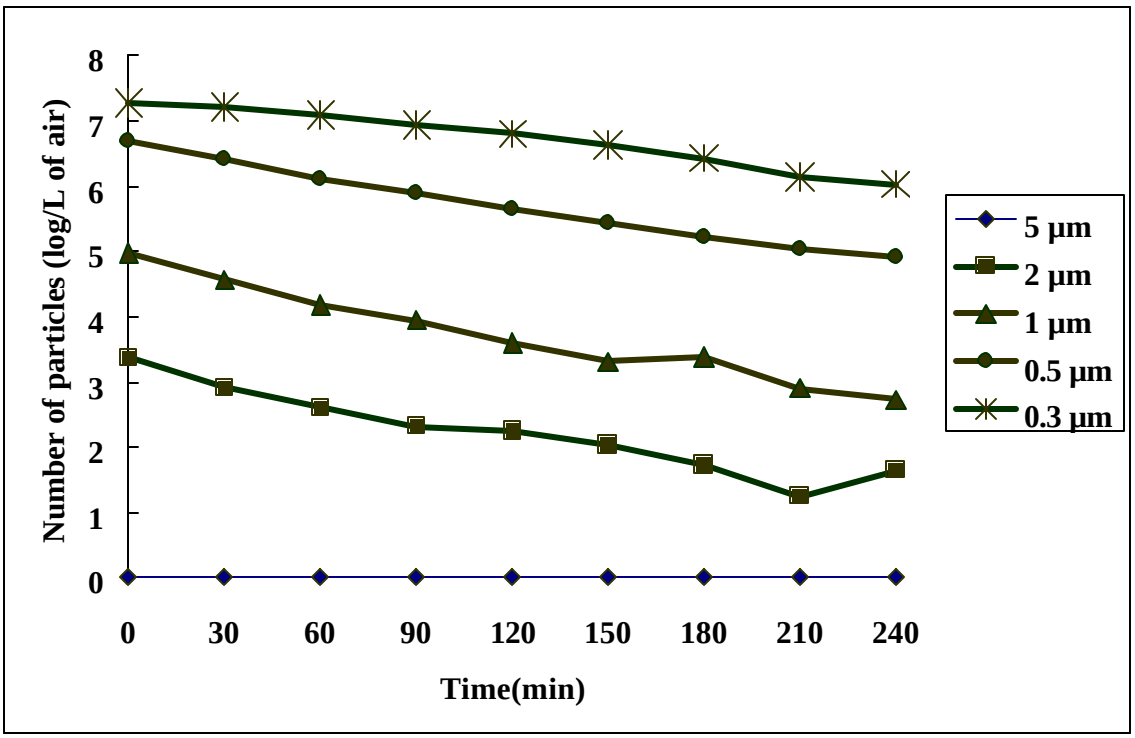

Fig. 4. Dynamics of sedimentation of particles in relation to their size when *J. denitrificans* was aerosolized in a bioaerosol containment chamber at 40% relative humidity and room temperature (22-23 ^{o}C)

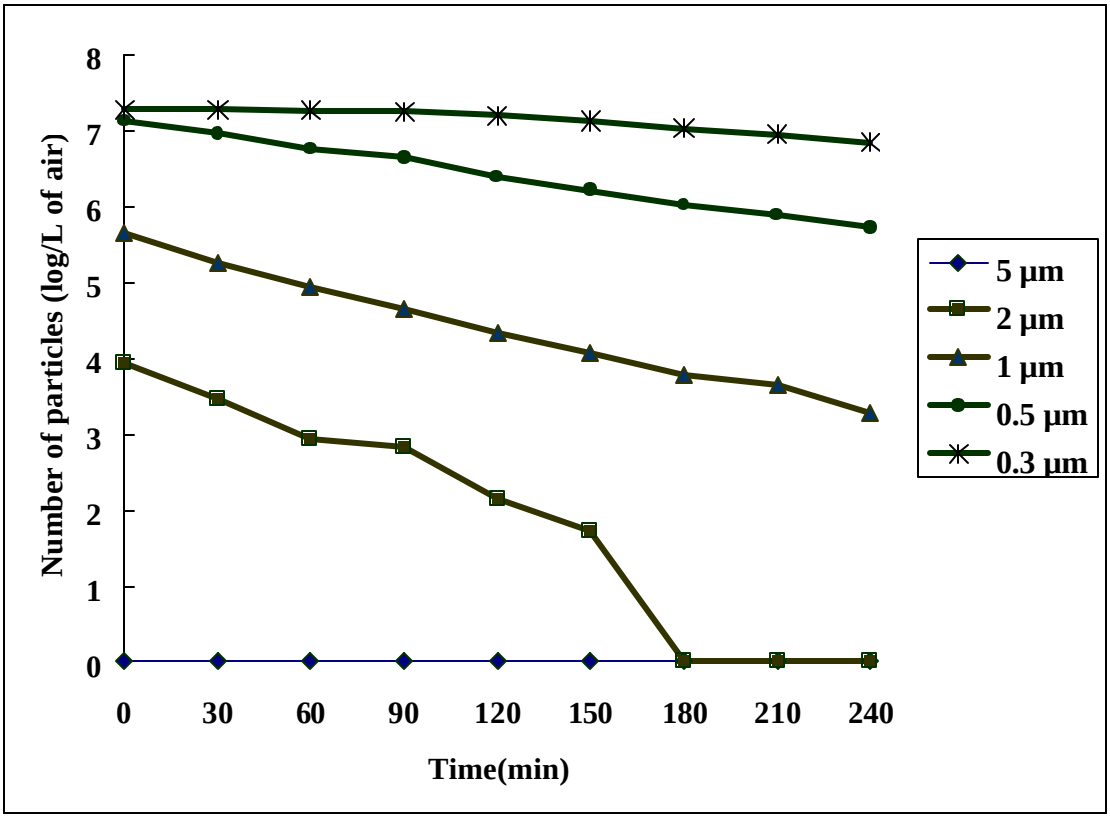

Fig. 5. Dynamics of sedimentation of particles in relation to their size when *J. denitrificans* was aerosolized in a bioaerosol containment chamber at 75% relative humidity and room temperature (22-23 ^{o}C)

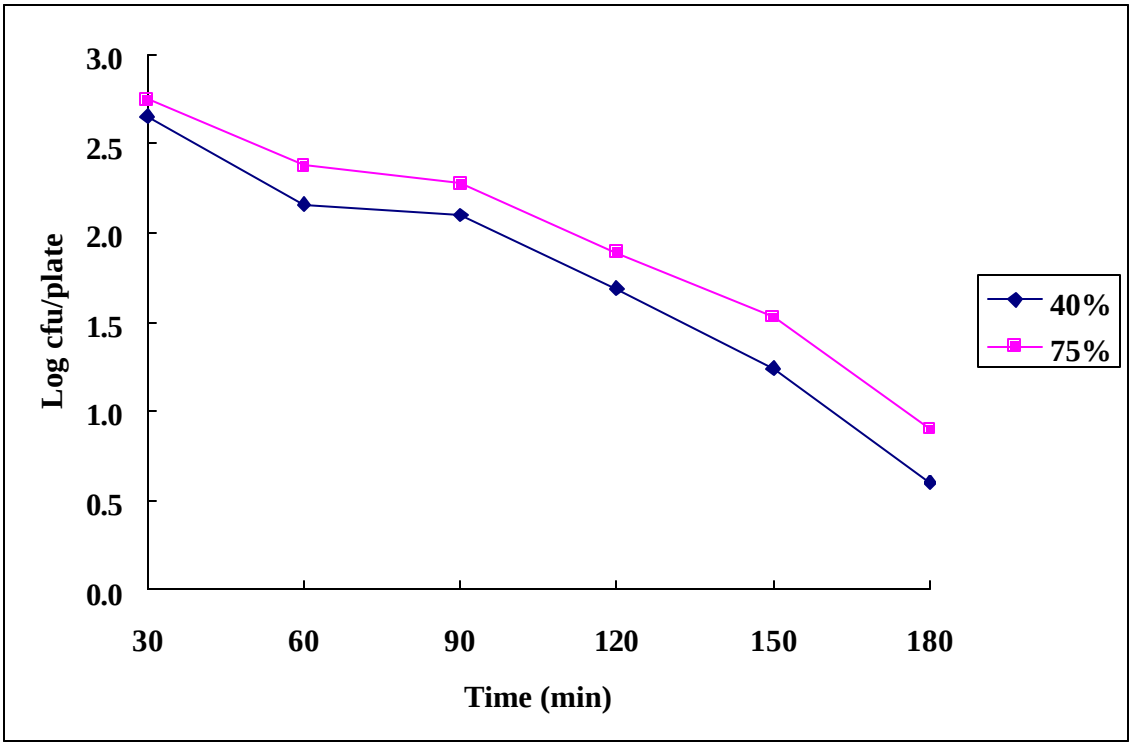

Fig. 6. Comparison of settling rates of *J. denitrificans* in a bioaerosol containment chamber at 38 and 75% RH and room temperature (22-23 ^{o}C)

Table 4. Number of *J. denitrificans* -positive samples on cured turkey ham following aerosolization in a bioaerosol containment chamber at room temperature (22-23°C) adjusted to 40 and 75% relative humidity (RH) for different exposure times.

40% RH

Initial cell number[a]	Exposure time (min)						
(cfu/L air)	Control	5	30	60	90	180	240
55	0/3[b]	0/3	0/3	0/3	0/3	0/3	0/3
$1.8x10^2$	0/3	0/3	0/3	0/3	0/3	0/3	0/3
$2.3x10^3$	0/3	0/3	0/3	0/3	1/3	0/3	1/3
$4.5x10^5$	0/3	2/3	3/3	3/3	3/3	3/3	3/3

75% RH

Initial cell number[a]	Exposure time (min)					
(cfu/L air)	Control	5	30	60	90	180
67	0/3	0/3	0/3	0/3	0/3	0/3
$2.5x10^2$	0/3	0/3	0/3	0/3	0/3	0/3
$3.2x10^3$	0/3	1/3	2/3	1/3	3/3	3/3
$3.5x10^5$	0/3	3/3	3/3	3/3	3/3	3/3

[a]Initial cell number of *J. denitrificans* introduced into air by a nebulizer.

[b]Number of *J. denitrificans* -positive samples/total number of meat samples at each exposure time. *J. denitrificans* was detected by culture enrichment.

Table 5. *J. denitrificans* cell numbers (cfu/three slices) on non-cured turkey meat following aerosolization in a bioaerosol containment chamber at room temperature (22-23°C) adjusted to 40 and 75% relative humidity (RH) for different exposure times.

40% RH

Initial cell number[a]	Exposure time (min)						
(cfu/L air)	Control	5	30	60	90	180	240
55	0	0	0	0	0	0	0
$1.8x10^2$	0	0	0	0	0	0	0
$2.3x10^3$	0	0	0	0	0	0	1
$4.5x10^5$	0	3	5	9	2	8	5

75% RH

Initial cell number[a]	Exposure time (min)					
(cfu/L air)	Control	5	30	60	90	180
67	0	0	0	0	0	0
$2.5x10^2$	0	0	0	0	0	0
$3.2x10^3$	0	1	4	0	8	2
$3.5x10^5$	0	2	8	12	7	11

[a]Initial cell number of *J. denitrificans* introduced into air by a nebulizer.

Table 6. *J. denitrificans* and *L. monocytogenes* cell numbers (log_{10}cfu/g) on non-cured turkey meat vacuum or not vacuum packaged and held at 4 and 12°C for 4 and 7 days.

Bacteria	*J. denitrificans*				*L. monocytogenes*			
Temperature	4°C		12°C		4°C		12°C	
Packaging	VC[a]	notVC[a]	VC	notVC	VC	notVC	VC	notVC
Initial inoculum	1.33	1.33	1.33	1.33	1.45	1.45	1.45	1.45
Day 4	ND[b]	ND	1.26	1.55	1.92	2.29	2.88	3.01
Day 7	ND	ND	0.33	0.43	3.96	4.07	5.88	5.01

[a]VC, vacuum-packaged; not VC, not vacuum-packaged.
[b]ND, not detected by direct plating or enrichment culture.

Phase III

Objective: Determine degree of product contamination in relation to distance and time of exposure following aerosolization of *J. denitrificans* in a pilot-scale poultry processing facility

Material and Methods

Bacterial strain. *Jonesia denitrificans* was used as a surrogate for *L. monocytogenes*. The culture was prepared according to the protocol described Phase II.

Enumeration and identification of *J. denitrificans*. Detection, enumeration, and identification procedures used were the same as those described in Phase II.

Aerosolization of *J. denitrificans* into a poultry processing facility. *J. denitrificans* was aerosolized into a pilot-scale poultry deboning facility at Auburn University (Attachment B). Three air conditioners of which each had five fans were used to chill the room. One day before *J. denitrificans* was introduced into the room, two of the air conditioners (AC units A and B) were started to reduce the interior temperature to 12°C and create a high airflow environment. One of air conditioners (AC unit C) was not used in order to provide an area with reduced airflow. The interior size of the room was 103,490,209 cm^3, an equivalent of 103,490 L, and *J. denitrificans* was aerosolized at 10^3 cfu/L air. Four nebulizers, each containing 5 ml of *Jonesia* suspension, were used to aerosolize *J. denitrificans*. The nebuizers were placed in front of the second and fourth fans of each operating air conditioner. The door of the deboning room was sealed with duct tapes for the aerosolization and the relative humidity was maintained at 60 to 70% throughout the study.

Environmental sampling and exposure of turkey meat. Twenty-eight environmental surface samples in the deboning room and 47 throughout the entire pilot plant were obtained by swab testing. Each surface area swabbed was 15 x 20 cm (300 cm^2). Environmental swab samples were obtained at one week and 4 hr prior to aerosolization, at the time of aerosolization, and 8 hr after aerosolization. Petri dishes containing TSAYE-CBA-MOX or turkey meat were placed on the floor to determine the degree of contamination and distribution of aerosolized *J. denitrificans*. Five rows of Petri dishes were positioned in front of each air conditioner. Each row contained 10 Petri dishes. TSAYE-CBA-MOX plates and turkey meat were alternated between each other in the same row. The first row of Petri dishes was positioned 50 cm away from the baseline (based on front edge of each air conditioner at floor level and each row of Petri dishes was 50 cm apart). The TSAYE-CBA-MOX plates and turkey meat were exposed for 3 hr. The experiment was repeated twice and results reported are the average of duplicate determinations.

Results

Environmental sampling and exposure of turkey meat. No *J. denitrificans* was recovered in environmental samples from the deboning room before or after aerosolization. The largest number of *J. denitrificans*-positive plates and turkey meat samples were obtained from the row 100 cm away from the air conditioners (Tables 7 and 8). *J. denitrificans* was not detected on turkey meat but was detected on one TSAYE-CBA-MOX plate positioned in front of the third air conditioner (AC unit C), which was not operating during the aerosolization studies.

Table 7. *J. denitrificans* cell numbers on TSAYE-CBA-MOX overlaid plates (cfu/plate) and number of *J. denitrificans*-positive turkey meat samples at different distances from air conditioning units 3 hours after aerosolizing the organism in a pilot-scale poultry deboning room.

Distance (cm) from AC	AC unit A		AC unit B	
	cfu/plate[a]	No. pos./No. tested	cfu/plate[a]	No. pos./No. tested[b]
50	49	1/5	25	1/5
100	267	2/5	325	3/5
150	198	0/5	125	0/5
200	155	1/5	213	1/5
250	89	0/5	156	0/5

[a]Reported cell number is the average cell number on five plates in each row.
[b]Number of *J. denitrificans*-positive meat samples/total number of meat samples (5) in each row.

Table 8. *J. denitrificans* cell numbers on TSAYE-CBA-MOX overlaid plates (cfu/plate) and number of *J. denitrificans*-positive turkey meat samples at different distances from air conditioning units 3 h after aerosolization of *J. denitrificans* in a small-scale poultry deboning room.

Distance (cm) from	AC unit A		AC unit B	
Air Conditioner	cfu/plate[a]	No. pos./No. tested[b]	cfu/plate[a]	No. pos./No. tested[b]
50	39	0/5	35	0/5
100	256	2/5	225	2/5
150	168	1/5	103	1/5
200	125	1/5	153	0/5
250	68	0/5	102	0/5

[a]Reported cell number is the average number on five plates in each row.
[b]Number of *J. denitrificans*-positive meat samples/total number of meat samples (5) in each row.

Attachment A

Bioaerosol Containment Chamber

1. Gloves
2. Pump for air circulation within chamber
3. Nebulizer for generation of bio-aerosols
4. Trays with saturated salt solution
5. Air filter

Attachment B

Arrangement of plates of turkey meat and TSAYE-CBA-MOX overlaid plates in a small-scale poultry deboning room in which *Jonesia denitrificans* was aerosolized.