MEASURING AND MODELING THE INFLUENCE OF RELATIVE HUMIDITY, TEMPERATURE, AND BUFFER TYPE ON THE SURVIVAL AND GROWTH OF *ENTEROBACTER AEROGENES*

by

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ABSTRACT OF THE THESIS

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Survival of bacteria on surfaces is an important part in understanding cross-contamination of food. The effects of temperature and relative humidity appear to play important roles in understanding how bacteria survive on surfaces. Surface type and inoculum matrix also appear to influence bacterial survival. This study examines how relative humidity, temperature, and inoculum matrix effected the survival of non-pathogenic *Enterobacter aerogenes* on common surfaces stainless steel, PVC, and ceramic tile. While surface type seemed to have little effect on survival, temperature showed a clear effect. *E. aerogenes* survived better at 7°C at 15 and 50% relative humidity on all surfaces. Inoculum matrix composition influenced survival and even allowed growth under some high RH conditions. Cells suspended in distilled water experienced a larger decrease in concentration immediately after inoculation on the surface vs. 0.1% peptone or 1% PBS. Cells suspended in 1% PBS showed a sharper decline in survival after 120 hours compared to 0.1% peptone both 15 and 50% relative humidity but cells in both matrices had similar tailing up to 3 weeks. Cells suspended in

0.1% peptone showed greatest growth and had the highest population density (~8 log CFU/ml) when the organism was inoculated into 10 mL of peptone. Cells suspended in PBS or distilled water showed ~2 log CFU/ml increase in concentration. When cells in 0.1% peptone were inoculated onto a stainless steel coupon and placed at 100% RH, concentration increased to ~7 log CFU/coupon after a lag time of ~24 hours while cells in 1% PBS increased to ~5 log CFU/coupon followed by a decline over 3 weeks. DMFit and GinaFit software could model inactivation on surfaces at all conditions other than 100% RH at 21°C.

Dedication

I'd like to dedicate this thesis to my loving parents John and Holly for always holding confidence in me, even when my own wavered and for their endless patience through all of my strenuous times. I'd also like to dedicate this thesis to my two sisters Reilly and Shannon from whom I draw inspiration from every day. And finally to the memory of my cousin Adam, one of the brightest and warmest people I've ever had the pleasure of getting to know.

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Literature Review

Food Contact Surfaces

Stainless steel is one of the most widely used surfaces in commercial kitchens and food processing facilities because it is easy to clean and will not corrode [1], and for this reason it is also commonly used in food microbiology research. Understanding bacteria adhesion to and survival on stainless steel surfaces is also an important factor in understanding and preventing foodborne outbreaks [2, 3]. Stainless steel has been shown to promote biofilm formation and allow microorganisms to survive longer vs. other metals, which can in turn promote cross-contamination in food processing facilities [1, 4, 5].

Ceramic tile is widely used as flooring in homes and food processing facilities. Ceramic tiles are typically hydrophilic which allow water to spread and which can create an antimicrobial environment [6]. The long term survival of pathogenic organisms on ceramic tile has been shown to be a potential cause of foodborne disease outbreaks [7].

Polyvinyl Chloride (PVC) is a thermoplastic that can be used in many ways in food facilities and produce packinghouses, including as a food contact surface [8]. Bottles, thermoformed foil, cling film, and lids or caps are some of the common uses in food packaging [8]. PVC-lined surfaces are also often used for drainage and piping systems as they will not corrode and are flexible, lightweight, and chemically resistant [9]. PVC is hydrophobic and may contain microscopic holes and crevices which can promote cracking over time and larger macroscopic holes and crevices to form [10].

Relative Humidity

The control of relative humidity (RH) has been increasingly viewed as an important factor in understanding the behavior of microorganisms and as a potential control for the spread of microorganisms [11-15]. Relative humidity is defined as the partial pressure of the water vapor in the air compared to the equilibrium vapor pressure of the air at a given temperature. Various salts can be dissolved into water and placed into a sealed environment (e.g. a glass desiccator) to control the relative humidity of that sealed environment [16]. Microorganisms on surfaces will experience desiccation at lower relative humidities, which inhibits their growth and reduces their metabolic activity [17-20]. This effect is similar to how bacteria may behave when present in a low moisture or low water activity food, although the chemical species in foods may make those interactions more complex [11, 21-23]. Microorganisms are unable to grow without the presence of water regardless of other environmental conditions [17], and a higher relative humidity has been shown to better support the growth and survivability of microorganisms in closed environments [15, 20, 24].

Temperature and Bacteria

Temperature has a clear effect on the survival of bacteria on surfaces and food where other conditions do not permit growth, and an increase in temperature will lead to an increase rate of inactivation and decrease in survival [25-27]. The same is true for the opposite effect and a decrease in temperature will cause a decrease in inactivation rate and promote survival [28-32].

The effect of temperature on the growth of bacteria is one of the most widely studied factors influencing bacterial behavior. The effect of temperature has been shown in many matrices, and generally speaking increased temperature allows for greater metabolic activity, which increases microbial growth rate [33, 34]. Bacteria grow more rapidly as temperature increases, and a decrease in temperature stops growth [33, 35]. Temperature has been repeatedly shown to have a significant effect pathogen growth rate in different foods [36-40].

Survival of Bacteria on Surfaces

Bacterial survival on surfaces can play an important role in cross contamination. Research has shown that inoculation size affects risk of cross contamination [41, 42]. Conditions that allow for greater survival potentially lead to a greater bacterial transfer. Surface physiochemistry research shows that bacterial survival and/or recovery from surfaces is influenced by surface pore size and hydrophobicity, as large pores absorb the inoculated bacteria which may decrease apparent survival and/or reduce recovery, while hydrophobic surfaces promote slower drying due to larger droplet sizes [10, 43-45]. Temperature has a clear effect on the survival of microorganisms on surfaces, as microbes survive worse at higher temperature [10, 27, 31, 44]. The composition of the substrate containing bacteria inoculated onto surfaces also affects survival [43, 44, 46-52]. The amount of moisture in the air, measured as relative humidity plays a role in survival on non-biologic surfaces, as does food water activity when measuring bacterial survival in food products [10-12, 24, 27, 53, 54]. The initial level of bacteria clearly effects survival time, as higher concentrations allow for longer survival (or time above detection limit) [47, 55]. Degree of attachment and biofilm formation have also been

shown to effect bacterial survival on surfaces [20, 61]. Some metal surfaces, (e.g copper) have overt antimicrobial effects, likely due to enzyme inhibition, production of free hydroxyl groups and blockage of protein binding sites [1, 5, 56-60].

Bacteria and Buffers

Peptone buffer is made from a solution of digested peptides and saline to create a pH of ~7.4 environment, at an appropriate osmotic pressure to promote bacterial survival with minimal nutrients. Peptone buffers have been used since at least the early 1900's [62]. The concentration of peptone in a buffer has shown to affect microbial survival on surfaces [46, 63]. Studies have evaluated the ability different peptone buffers to recover an subsequently culture pathogens like *Escherichia coli* and *Salmonella* from foods [63-65]. Peptone water has been shown to increase the recovery of damaged and desiccated cells [65].

Phosphate buffered saline (PBS) is made from sodium phosphate and sodium chloride to create an environment where the osmatic pressure is approximately equal to that of the osmotic pressure inside the cell and a pH of ~7.4. *Salmonella* survival on surfaces has been shown to be greater when suspended in tryptic soy broth (TSB) rather than when suspended in PBS, due to the greater nutrient concentration in TSB [43]. Studies have shown that *Pseudomonas spp., Listeria monocytogenes*, and *Salmonella* can survive for over 30 weeks while suspended in PBS [66]. PBS containing added minimal nutrients (sodium citrate, lactic and malic acids) has been shown to allow for the growth of *Pseudomonas* and *E. coli* [67].

Distilled water is not commonly used to create bacterial suspensions for laboratory experiments as it can cause cell lysis due a change in osmotic pressure [68, 69], leading

to the use of peptone buffer or PBS as mentioned above. While bacteria placed in water can lyse, the ability of bacterial pathogens to survive in drinking water for extended periods demonstrates that reports of bacterial death in water may be greatly exaggerated. Studies have been conducted to understand the ability for microorganisms to survive in distilled and drinking water [68, 70, 71], with widely varying results ranging from less than a 1 log decrease over 5 days for bacteria isolated from lake water, a 2 log decline in 24 hr for bacteria subcultured on blood agar plates, and a 1 log decline in 1 hr when bacterial cells were transferred from foods to distilled water.

Characteristics of Bacteria Influencing Survival

Biofilms are groupings of bacteria cells held together by extracellular polymeric substances [75, 76] and the ability for form a biofilm is known to potentially influence bacterial survival. Biofilms are thought to be a survival mechanism when bacteria are attached to surfaces, as bacteria show better survival in biofilms vs. in suspension [61, 77-79]. Bacteria in biofilms show reduced growth and changes in gene expression [76, 79, 81]. Biofilm formation has been shown to contribute to long-term survival of pathogens in low-moisture environments that can eventually lead to cross-contamination of foods [78, 82-85]. Biofilms can also lead to increased surface corrosion [80], reduced cleaning effectiveness and poor removal of bacteria from surfaces [76, 86-88]. Bacterial attachment or adhesion can be an important factor in bacterial survival on surfaces and subsequent risk of cross-contamination. Adhesion is a necessary first step in biofilm formation, which is important for reasons discussed above [84, 86, 88]. Bacterial adhesion to surfaces can be affected by contact time, temperature, moisture, and surface free energy [35, 89-91]. Attachment is also affected by the cells ability to form fimbrae

and flagella, as well as cell surface hydrophobicity [75, 76, 92, 93]. Microorganisms have been shown to attach more readily to hydrophobic, non-polar surfaces like Teflon or plastic vs. metals [93-97]. Pathogens can readily attach to common kitchen surfaces then transfer to foods leading to illness [2].

Predictive Modeling of Bacterial Survival and Growth

Predictive models can be a useful way of saving the time and expense of laboratory experiments. Microbial models are normally created from laboratory data measuring change in bacterial concentration over time. Conventional wisdom holds that bacterial inactivation follows a log linear pattern [100]. More recently models have used other shapes to describe bacterial survival curves. These include the Weibull and Biphasic models which can describe survival curves with "tails" where the rate of decline slows over time [101, 102]. GinaFit and DMFit are two freely available tools which a variety of log-linear and non-log-linear models [103, 104] Tri-phasic models which describe microbial populations that experience a shoulder, decline and tailing, can also be fit [104]. Survival models can be used for microbes on surfaces, to improve the understanding of the risk of cross contamination [11, 48, 49] as well as for pathogens in foods [23, 26, 105-108]. All such models can be used to create secondary models or in microbial risk assessment as an aid to risk management [23, 108, 109].

Predictive growth models are similarly created from bacterial concentration data, where those data show an increase in concentration over time. Models for the growth of pathogens in foods can be important tools for risk managers [110]. Many growth models have historically been created from experiments conducted in laboratory media [34, 111, 112], while more recently developed models have been created from experiments in

foods [113-115]. Factors that are considered in many of these models include, pH, water activity, acidulant [112], temperature [38, 116], starting bacterial concentration or food type [111]. The Gompertz, Baranyi and Roberts, linear, and tri-phasic models have all been used extensively to predict bacterial growth [104, 111, 116-118]. As mentioned in the section above, DMFit can be used to model bacterial behavior [104]. The DMfit growth modeling tools can fit the Baranyi and Roberts and log linear models to growth data. Secondary models can be created which describe the effect of factors like temperature, pH and water activity on bacterial growth curves [112, 114, 116]. Combase predictor is an online tool containing many such secondary tools [119]. As noted above, growth models can be used in quantitative risk assessment to improve prediction and guide risk management decisions [109, 110, 120].

Summary

Cross-contamination from food contact surfaces to foods can spread pathogenic organisms in homes, restaurants and food processing facilities. The survival of microorganisms on foods, and contact surfaces can lead to foodborne disease. Our understanding of the relationships between the inoculation method and matrix, time, temperature, relative humidity, and surfaces characteristics is poorly understood. An improved understanding of these inter-relationships will benefit future experimental design, mathematical model development, and future risk-based decision making.

Measuring and Modeling the Influence of Relative Humidity, Temperature, and Buffer Type on the Survival and Growth of *Enterobacter aerogenes*

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Abstract

Survival of bacteria on surfaces is an important part in understanding cross-contamination of food. The effects of temperature and relative humidity appear to play important roles in understanding how bacteria survive on surfaces. Surface type and inoculum matrix also appear to influence bacterial survival. This study examines how relative humidity, temperature, and inoculum matrix effected the survival of non-pathogenic *Enterobacter aerogenes* on common surfaces stainless steel, PVC, and ceramic tile. While surface type seemed to have little effect on survival, temperature showed a clear effect. *E. aerogenes* survived better at 7°C at 15 and 50% relative humidity on all surfaces. Inoculum matrix composition influenced survival and even allowed growth under some high RH conditions. Cells suspended in distilled water experienced a larger decrease in concentration immediately after inoculation on the surface vs. 0.1% peptone or 1% PBS. Cells suspended in 1% PBS showed a sharper decline in survival after 120 hours compared to 0.1% peptone both 15 and 50% relative humidity but cells in both matrices had similar tailing up to 3 weeks. Cells suspended in 0.1% peptone showed greatest growth and had the highest population density (~8 log CFU/ml) when the organism was inoculated into 10 mL of peptone. Cells suspended in PBS or distilled water showed ~2 log CFU/ml increase in concentration. When cells in

0.1% peptone were inoculated onto a stainless steel coupon and placed at 100% RH, concentration increased to ~7 log CFU/coupon after a lag time of ~24 hours while cells in 1% PBS increased to ~5 log CFU/coupon followed by a decline over 3 weeks. DMFit and GinaFit software could model inactivation on surfaces at all conditions other than 100% RH at 21°C.

Introduction

Cross contamination of foods by pathogens present on contaminated surfaces can lead to foodborne illness, thus the survival of microorganisms on food contact surfaces is an important part of understanding cross-contamination risk. Many pathogenic organisms including *Escherichia coli* and *Salmonella* can survive for long periods of time on non-biological surfaces [5, 10, 48, 53, 55]. Temperature [10, 11, 27, 31, 44], relative humidity (RH) [11, 14, 20], surface type [10, 43, 44] and microbial matrix [46, 48-50] all appear to play a role in bacterial survival on surfaces. While some studies have documented the effect of temperature on bacterial survival, little research has addressed the impact of relative humidity on survival, especially in interaction with temperature. Stainless steel, ceramic tile, and polyvinyl chloride are common surfaces found in homes, restaurants and food processing facilities. Surface free energy, hydrophobicity and porosity have all been seen to effect bacteria attachment ability and biofilm formation, which can be important factors in bacterial survival on surfaces [2, 75, 76, 89, 91, 97, 121, 122]. Understanding survival of bacteria on these surfaces can help to create a better understanding of when cross-contamination can occur to help manage the risk foodborne disease [123]. Predictive primary and secondary models for growth and survival have been incorporated into risk assessments to better understand likelihood of exposure [108-110, 120]. Advancement in the use of predictive survival models may assist in incorporating models for cross-contamination into quantitative microbial risk assessment.

Materials and Methods

Preparation of Surfaces

Stainless steel (0.018” thickness, 16 gauge; onlinemetals.com, Seattle, WA), polyvinyl chloride (1/8” thickness; McMaster-Carr, Robinsville, NJ), and ceramic tile (Brancacci Windrift Beige, Daltile, Dallas, TX) were purchased online or locally and cut to 5cm x 5cm tiles. Tiles were sprayed with 70% ethanol, allowed to air dry, wrapped in aluminum foil, autoclaved, sprayed again with 70% ethanol and allowed to dry prior to inoculation.

Preparation of Bacterial Strains

Enterobacter aerogenes strain B199A, a non-pathogenic microorganism that has previously shown similar attachment characteristics as *Salmonella* to chicken skin was used for all experiments (Vivolac Cultures, Indianapolis, Ind) [98]. This strain is resistant to nalidixic acid and control experiments showed that no nalidixic acid resistant *E. aerogenes* were found above the detection limit on any surfaces after disinfection. Cultures were prepared in a similar method that has been done previously [98, 99]. A frozen stock of *E. aerogenes* in 80% glycerol solution was streaked onto tryptic soy agar (Difco, BD, Sparks, MD) containing 50 µg/ml of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.), referred to as TSA-na). One colony was grown overnight in 10 ml tryptic soy broth (Difco, BD, Sparks, MD) containing 50 µg /ml of nalidixic acid and incubated at 37°C for 24 hours. Inoculum matrices were of three different types as described below. Cells were harvested from the overnight culture in TSA-na by centrifuging at 5,000 x g for 10 min, and washed twice in either 0.1% peptone solution (Difco, BD), 1% phosphate buffered saline solution (Difco, BD), or sterile distilled water. A final concentration of 10^8 CFU/ml was verified on TSA-na plates for each matrix.

Preparation of Controlled Environment

Lithium chloride or potassium carbonate (each 230 g) were slowly mixed into 100 ml of water while being heated slightly to create saturated salt solutions for 15 and 50% RH respectively. Potassium sulfate salt (250 g) was mixed into 100ml of water to create the 100% RH environment. Salt solutions were placed in the bottom of desiccators and given 24 hours for the relative humidity to stabilize. Vaseline was used around the rim of the desiccator to promote a water tight seal. Data loggers (LASCAR Electronics, Erie, PA) for RH and temperature were used to monitor the environment. Loggers were sensitive to 0.5 (+/- 1) °C and 1(+/-2) % RH.

Survival Based on Surface and Temperatures

Three surfaces types (stainless steel, PVC, and ceramic tile) were inoculated with 100 µl containing 10^8 CFU/ml 0.1% peptone and allowed to dry for approximately 2 hours for an initial concentration of ~10^7 CFU per coupon. Coupons were then placed in desiccators containing saturated salt solutions at 15, 50 or 100% relative humidity. Desiccators were placed either on the bench top (21 °C) or in a walk-in cooler (7 °C). Tiles were removed the desiccator at 10 time points (0s, 8h, 1d, 2d, 3d, 5d, 7d, 10d, 14d, 21d). Each coupon was placed in a sterile 207 ml Whirl-Pak sampling bag (Nasco, Fort Atkinson, WI) with 10 ml of 0.1% peptone solution. The rub-shake method was used for 1 minute to fully detach the microorganisms from the surfaces [10]. Dilutions were plated on TSA-na plates and incubated at 37°C for 24 hours and colonies were counted. Populations were expressed in CFU per surface.

Survival Based on Matrix Type

Survival of *E. aerogenes* was then tested on only stainless steel surfaces with different matrix types. As described above, cultures were washed with either 0.1% peptone, 1%

PBS or sterile distilled water and inoculated onto stainless steel surfaces and placed in a desiccator containing saturated salt solutions at either 15, 50, 100% relative humidity. Desiccators were placed on the lab benchtop (21°C), and tiles were sampled at 10 time points (0s, 8h, 1d, 2d, 3d, 5d, 7d, 10d, 14d, 21d) for peptone and PBS samples and over 10 more frequent time points (0h, 4h, 8h, 12h, 24h, 36h, 48h, 72h, 120h, 168h) for sterile distilled water samples. Surfaces were placed in sterile Whirl-Pak bags containing 10ml of the same matrix that was used for inoculation, and the rub-shake method as previously described was used to detach microorganisms from surface then diluted and plated on the TSA-na plates. Colonies were counted and expressed as log CFU per surface.

Growth in Different Buffers

Sterile centrifuge tubes (2 ml, Thermo Fisher Scientific, Waltham, MA) were used to hold different matrices for growth of bacteria. *E. aerogenes* was inoculated into the centrifuge tubes at concentrations of ~2, 4, 6 and 8 CFU/ml in each diluent (0.1% peptone, 1% PBS, and sterile distilled water). Centrifuge tubes were capped and held at 21 °C and samples at 6 time points (0h, 4h, 8h, 1d, 2d, 3d) for peptone and PBS or 6 more frequent time points (0h, 8h, 1d, 2d , 3d, and 5d) for distilled water. Tubes were vortexed for 30 seconds to distribute bacteria in the matrix prior to sampling. Samples were plated on TSA-na and colonies were counted and transformed to CFU/ml.

Survival at Different Starting Concentrations at a High Humidity

E. aerogenes was inoculated onto stainless steel coupons at starting concentrations of ~2, 4, 6 log CFU/surface with 0.1% peptone and 1% PBS and placed in desiccators containing saturated potassium sulfate salt solutions to insure 100% RH. Coupons were removed from the desiccators at 10 time points (0s, 8h, 1d, 2d, 3d, 5d, 7d, 10d, 14d, 21d).

Each coupon was placed in a sterile Whirl-Pak bag with 10 ml of 0.1% peptone or PBS solution. The rub-shake method as previously described was again applied for 1 min to fully detach the bacteria from the surfaces as described in the previous section. Dilutions were plated on TSA-na plates and incubated at 37°C for 24 hours and colonies were counted. Populations were expressed in CFU per surface.

Data Analysis

Predictive models were created using the Microsoft Excel add-in tool GinaFit [103] (Katholieke Universiteit Leuven, Leuven, Belgium) and DMfit [104](Institute of Food Research, Norwich, UK). GinaFit was used for inactivation models while DMfit was used for growth and inactivation models. Statistical analysis for standard deviation and variance was done using Excel (Microsoft, Redmond, WA).

Results

Survival Based on Surface and Temperatures

E. aerogenes survival generally showed a decline followed by a plateau, and could be modeled using log linear, Biphasic, Weibull, or Baranyi and Roberts models. R^2 values for the primary models for experiments conducted at 15 and 50% relative humidity ranged from 0.73 to 0.96 indicating relatively good fit (Table 1 and 2). Goodness of fit values for survival at 100% RH were significantly lower, indicating the inability to use this data fitting model for these conditions (Table 1 and 2). Final concentration of *E. aerogenes* at 7°C were 4.5, 4.9 and 5.0 log CFU/surface after 21 days at 15, 50 and 100% RH for stainless steel, 5.1, 5.4 and 5.5 log CFU/surface after 21 days at 15, 50, and 100% RH for PVC tiles and 4.7, 5.2and 5.9 log CFU/surface at 15, 50 and 100% RH for ceramic tiles respectively (Figure 1). *E. aerogenes* generally did not survive as well at

21°C with final concentrations of 1.3 and 2.7 CFU/tile at 15 and 50% RH respectively, but showed either growth or injury recovery at 100% RH, with a final concentration of 6.5 log CFU/surface for stainless steel tiles, with final concentrations after 21 days of 3.4, 3.2 and 7.0 log CFU/surface for PVC tiles at 15, 50, and 100% RH and 2.8, 3.2, and 6.7 log CFU/surface at 15, 50, and 100% RH after 21 days for ceramic tiles (Figure 1).

Survival Based on Matrix Type

As shown in the previous sections *E. aerogenes* in 0.1% peptone declined to final concentrations of 1.3 and 2.7 CFU/tile at 15 and 50% RH respectively, but showed either growth or injury recovery at 100% RH, with a final concentration of 6.5 log CFU/surface for stainless steel tiles (Figure 2). Final concentrations of *E. aerogenes* at 21°C were 1.2 and 1.5 log CFU per surface at 15 and 50% RH while there was very little reduction at 100% RH as there was a final concentration of 6.7 log CFU per surface when using 1% PBS (Figure 2). *E. aerogenes* showed a more rapid decline after 120 hours when using 1% PBS to 2.7 and 2.5 log CFU/coupon at 15 and 50% RH as compared to 3.5 and 4.3 log CFU/coupon at 15 and 50% RH for 0.1% peptone. When using the sterile distilled water as the matrix there was a greater reduction after the initial 2 hours drying on stainless steel from an initial concentration of ~5 log CFU at each RH and there was a more rapid reduction in the bacteria on the surfaces. This resulted in final concentrations of 1.6, 1.6, and 2.9 log CFU per surface after only 7 days (Figure 2). Model fit values can be found on Table 3 for DMFit and Table 4 for GinaFit. DMFit was able to model survival adequately for all conditions other than at a RH of 100% based on R^2 values of greater than 0.76 (Table 3). Survival using distilled water had an R^2 value of 0.68 while peptone and PBS showed negative R^2 values (Table 3). Models using GinaFit showed a

similar trend as survival at 100% RH was not adequately modeled based on R^2 and RSME values (Table 4).

Growth in Different Buffers

E. aerogenes grew best from the lowest starting concentrations in 0.1% peptone increasing from ~2, 4, and 6 log CFU to the maximum population density of ~8 log CFU/ml in about 2 days (Figure 3). The starting concentration of ~8 log CFU showed no change over 3 days. *E. aerogenes* showed either no change or a modest increase in concentration in PBS. Starting concentrations of ~2 and 4 log CFU grew to a maximum of 5.5 log CFU each after 3 days in PBS, while starting concentrations of ~6 and 8 CFU showed no change over 3 days (Figure 3). *E. aerogenes* in distilled water showed the least increase with the lowest starting concentration of ~2 log increasing to 4.5 log CFU after 5 days (Figure 3). The ~4 log starting concentration showed a slight increase to a maximum of 5.5 log CFU (Figure 3). *E. aerogenes* in distilled water at the ~6 and 8 starting concentrations showed no change over 3 days (Figure 3). The Baranyi and Roberts model did a good job of fitting the data for bacterial growth in 0.1% peptone at the starting concentrations of 2, 4, and 6, and these models all had R^2 values greater than 0.96 and SE of fit of less than 0.18 (Table 5). DMFit models for the highest starting concentration for peptone had an R^2 value of only 0.37 (Table 5). DMfit was able to model the growth in PBS at low starting concentrations of ~2 and 4 log CFU and these models had R^2 values of 0.95 or greater with SE of fit values below 0.11 (Table 5). The higher starting concentrations of ~6 and 8 log CFU were not well fitted using DMfit and these models had R^2 values of only 0.69 (Table 5). A similar pattern was seen when DMfit was used to model the data for the growth in distilled water. The lower two

starting concentrations were well fit with R^2 values of greater than 0.97 while models for the higher two starting concentrations had R^2 values of less than 0.47 (Table 5).

Survival at Different Starting Concentrations at a High Humidity

When *E. aerogenes* was inoculated onto the stainless steel surface and incubated at 21°C and 100% RH at a starting concentration of ~6 log CFU it displayed an interesting pattern of decreasing to 4.9 log CFU per surface after 24 hours to then increasing back to 6 log CFU per surface after 21 days (Figure 4). The two lower starting concentrations of ~2 and 4 showed similar characteristics with a lag or decline for 24 hours followed by an increase to a maximum of ~7 log CFU after 72 hours followed by a tailing effect through 21 days (Figure 4). When *E. aerogenes* was inoculated onto surfaces in PBS and incubated at 21°C at 100% RH there was no real dramatic change in population as *E. aerogenes* maintained a concentration of 6-7 log CFU over the 21 days (Figure 4). When a lower starting concentration was used, an increase was seen after 8 hours. With a starting concentration of ~3 log CFU the population increased over 120 hours to a maximum population of ~5 log CFU followed by a decline to 2.8 log CFU after 21 days (Figure 4). A similar pattern was seen with a starting concentration of ~1 log CFU where there was an increase to ~5 log CFU over 14 days followed by a decline to a final concentration of 4 log CFU after 21 days (Figure 4).

Discussion

Survival Based on Surface and Temperatures

Our results show that the surface types studied had no substantial influence on the survival of microorganisms despite differences in pore sizes and hydrophobicity [10, 75, 124]. Our results do clearly show the potentially complex interactions between

temperature and RH on survival of microorganisms. Our results are in agreement with other published research that shows that temperature clearly impacts bacterial survival on surfaces and lower temperatures result in longer survival times [10, 11, 27, 31, 44]. Our results support the observation that RH has little effect on survival at lower temperatures [11]. *E. aerogenes* survived better at 21 °C in higher humidities environments, most likely because increased atmospheric moisture gives a less stressful environment [20]. There was little difference between *E. aerogenes* survival at 15 and 50% at 21°C most likely because both these RH conditions are extreme enough to effect cell function [3, 11, 19, 20]. More research is clearly needed to understand the physiology of bacteria on the surfaces at high (~100%) RH and warm (21°C) conditions.

Predictive models were able to accurately describe the survival curves of this organism at all conditions other than 100% RH at 21°C on all surfaces, based on model adequacy values (Table 1 and 2). The Weibull and Biphasic model both showed the best fits based on R^2 and RMSE statistics. The Weibull model has greater parsimony since it has one fewer parameter than the Biphasic model and has the additional advantage of being used in several other survival studies allowing for stronger comparison [22, 23, 48, 49, 124].

Survival Based on Matrix Type

Survival curves of *E. aerogenes* at 15 and 50% followed a similar shape, with an initial sharp linear decline, followed by a tailing effect for all three buffer types (Figure 3). These effects are likely similar as discussed above because both RH values are similarly extreme and stressful to cell functions. Other research has also shown that matrix type and solute concentration does affect how organisms survive in desiccated states on surfaces [48-50]. While solute concentration has been shown previously to affect

inactivation rate of microorganisms during survival studies [43, 46], our results found little difference between 1% PBS and 0.1% peptone (Figure 3). This could be because the difference in available nutrients or osmotic pressure is so minimal between the two that once the organism has become desiccated the differences do not matter. Our models clearly show that experiments in peptone and PBS show a similar kmax values for rate of decline, while distilled water has a greater kmax value, indicating faster decline (Table 4). *E. aerogenes* survival patterns changed once RH increased to 100% and there was either no reduction (1% PBS) or decline followed by recovery (0.1% peptone). We believe that in the 100% RH experiments that condensation of moisture onto the surfaces created a rehydrating effect for both the dried PBS and peptone solutions creating an environment promoting long term bacterial survival [66]. Distilled water was clearly a more stressful environment leading to lower initial concentrations and subsequently shorter survival time but with similarly shaped survival curve albeit with a greater reduction rate (Table 3 and 4). Distilled water has been shown to potentially lyse cells because of changes in osmotic pressure, which is why buffers, salts or proteins are traditionally added to promote survival [67, 68]. Our experiments which show that *E. aerogenes* can survive on surfaces suspended in just distilled water is notable for two reasons: first it shows that bacteria can survive on surfaces for some time even in the absence of any nutrients and do constitute a potential for cross-contamination; and second that distilled water may be a preferred matrix for inoculation of bacteria onto surfaces in high RH conditions since other matrices seem to promote growth. More RH should be tested to fully understand the effect that RH has on bacteria survival, but experiments using distilled water may give a more accurate picture of how RH effects bacterial

survival, as the lack of salts or proteins seem to limit experimental artifacts with other systems at high RH conditions.

DMFit software modeled the PBS and peptone survival at 100% RH as growth as indicated by the positive maximum rate values (Table 3), while the GinaFit software showed a positive rate for the log linear model and was unable to model using the Biphasic and Weibull (Table 4). The Weibull and Biphasic model both showed the best fits based on various model adequacy statistics. As noted above, the Weibull model is more parsimonious that the Biphasic model and allows for easily compared to other survival studies which also used this model [22, 23, 48, 49, 124].

Growth in Different Matrices

Matrix type has shown a strong effect on the growth of bacteria, and a greater concentration of nutrients (up to a point) will lead to more rapid growth and higher final concentrations [125]. The 0.1% peptone buffer matrix showed the greatest ability to support bacterial growth in our experiments (up to ~8 log CFU/ml). All starting concentrations below this level were able to grow to the ~8 log CFU population limit in ~24 hours. The ability for bacteria to grow in 1% PBS was initially surprising but has also shown in some studies [66, 67]. Our results agreed with these studies that show that buffer containing a minimal concentration of minerals at an appropriate pH can support bacterial growth [70, 126]. The *E. aerogenes* cells in 1% PBS did require a longer time to reach and overall lower maximum population density (~5 log CFU/ml) compared to 0.1% peptone. This is not unexpected since 1% PBS represent a harsher, less nutrient dense environment compared to 0.1% peptone. The most surprising results were the population increases seen when *E. aerogenes* were inoculated into sterile distilled water. Distilled

water was able to support the lowest concentration of *E. aerogenes* (~4 log CFU/ml) achieved with the slowest overall growth rate, with cells at the lowest starting concentration requiring 5 days to reach this population density. We hypothesize two possible explanations for this startling result. The first is that there are enough nutrients left in the water to support growth even after the distilling process [127]. Studies have shown that tap, lake, and drinking water contain enough available carbon and other required nutrients to support bacterial growth [72, 129-135]. The second is that we are observing a cannibalization effect, where cells that survive the initial die-off are able to "cannibalize" nutrients from cells that died [67, 128]. Further research into concentrations of potential nutrient in the water after the distilling process, and experiments to explore this cannibalization effect should be conducted.

Survival at Different Starting Concentrations at High Humidity

There is a clear effect on bacterial survival when surfaces are stored at 100% RH [20]. There was some effect when the highest concentration of *E. aerogenes* were inoculated onto the stainless-steel surfaces in 0.1% peptone, as there was a decrease and then an increase after a few days. *E. aerogenes* increased to a concentration that was equivalent to the maximum population density, which, in this case, is ~7 log CFU/surface [136-139]. These results showed the need to test a lower starting concentration of *E. aerogenes* in an effort to determine whether the results represented injury and recovery or true growth. When *E. aerogenes* in 0.1% peptone were inoculated at concentrations of ~2 and 4 log CFU per surface there was a clear increase (i.e. >1 log increase) after only 24 hours of lag or decline (Figure 4). Experiments in liquid 0.1% peptone (not surfaces) we also observed an increase in concentration similar to what was seen on surfaces (Figure 3).

These results suggest that 0.1% peptone provides enough nutrients for *E. aerogenes* growth on the surface as long as the high RH conditions prevent dehydration. We observed clear moisture condensation on the surface of the stainless steel coupon at the inoculation site that would suggest that RH from the atmosphere is being pulled to this site creating conditions that allow *E. aerogenes* to grow [19]. The increase from the lower *E. aerogenes* starting concentrations in the 1% PBS inoculum was similar to that seen in 0.1% peptone: a lag period of a few days, followed by an increase to the (albeit lower) maximum population density. The difference seen in 1% PBS is that after the maximum population density is reached, the population in 1% PBS declines (Figure 4), unlike in 0.1% peptone for reasons that likely related to the more minimal nature of the 1% PBS matrix.

Summary

It has become increasingly important to understand the survival of microorganisms on foods, and food contact surfaces to aid in reducing foodborne disease. Our understanding of the relationships between the inoculation method and matrix, time, temperature, relative humidity, and surfaces characteristics is poorly understood. An improved understanding of these inter-relationships will benefit future experimental design, mathematical model development, and future risk-based decision making. This research has examined how relative humidity, temperature, and inoculum matrix effected the survival of non-pathogenic *Enterobacter aerogenes* on surfaces and has revealed some interesting findings that need to be replicated with other bacteria and viruses. While surface type seemed to have little effect on survival, temperature (as expected) showed a clear effect with better survival at 7°C than 21 °C. Survival patterns at 100% RH were

dramatically different that survival at 15% and 50% RH. Weibull and Biphasic models could successfully describe inactivation on surfaces at all conditions other than 100% RH at 21°C. Inoculum matrix composition influenced survival and even allowed growth under some 100% RH conditions in 0.1% peptone and 1% PBS when cells were inoculated at low levels onto stainless steel. This finding has important implications for microbiologists doing experiments with surface inoculation onto foods or food contact surfaces because it shows that depending on the inoculum matrix used, if RH is high or uncontrolled, growth may be observed because of the effect of the inoculum matrix itself. Subsequent experiments where *E. aerogenes* was inoculated into 0.1% peptone, 1% PBS and distilled water all showed the ability of the organism to increase in concentration to a degree that would typically be regarded as growth. Further research is needed to determine if the apparent growth in distilled water is due to available nutrients, cannibalism by surviving cells, or is not true growth but rather an artifact of the method.

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Table 1. Regression Parameters and Goodness-of-Fit values for the Baranyi and Roberts with no lag model using DMFit software for survival on different surfaces at different temperatures.

Temperature	Surface	RH	R-square:	SE of Fit:	Initial value	Maximum Rate	Final Value
21°C	SS	15%	0.909	0.47	5.959 ± 0.277	-0.0203 ± 0.00399	2.358 ± 0.273
		50%	0.809	0.505	5.998 ± 0.295	-0.0139 ± 0.00407	3.432 ± 0.298
		100%	-0.109	0.587	5.58 ± 0.325	0.00197 ± 0.00353	6.08 ± 0.421
	PVC	15%	0.815	0.436	6.194 ± 0.243	-0.0099 ± 0.0028	3.884 ± 0.297
		50%	0.768	0.502	6.257 ± 0.37	-0.0352 ± 0.0134	3.911 ± 0.218
		100%	0.218	0.892	5.427 ± 0.482	0.00601 ± 0.00487	7.0186 ± 0.658
	Tile	15%	0.868	0.414	6.174 ± 0.251	-0.016 ± 0.00405	3.6 ± 0.232
		50%	0.73	0.498	5.664 ± 0.245	-0.00599 ± 0.0017	3.184 ± 0.582
		100%	0.412	0.448	5.542 ± 0.27	0.00624 ± 0.00425	6.569 ± 0.253
7°C	SS	15%	0.784	0.277	6.305 ± 0.211	-0.0256 ± 0.00928	4.927 ± 0.114
		50%	0.962	0.0929	6.195 ± 0.0667	-0.0158 ± 0.00213	5.0321 ± 0.0413
		100%	0.528	0.346	6.184 ± 0.168	-0.00266 ± 0.00113	4.964 ± 0.541
	PVC	15%	0.858	0.221	6.519 ± 0.12	-0.00542 ± 0.00125	5.135 ± 0.159
		50%	0.893	0.146	6.738 ± 0.111	-0.0198 ± 0.0048	5.659 ± 0.0603
		100%	0.836	0.265	6.753 ± 0.202	-0.0282 ± 0.00867	5.206 ± 0.11
	Tile	15%	0.923	0.19	6.419 ± 0.11	-0.00842 ± 0.00148	4.826 ± 0.113
		50%	0.819	0.194	6.561 ± 0.158	-0.0243 ± 0.00958	5.47 ± 0.0776
		100%	0.614	0.355	6.33 ± 0.25	-0.0148 ± 0.00728	5.159 ± 0.159

Table 2. Regression parameters for the linear, biphasic, and Weibull models using GinaFit software for survival on different surfaces at different temperatures.

Temp	Surface	RH		kmax	f	kmax1	kmax2	delta	p	LOG10(N0)
21°C	SS	15%	Linear	0.020	-	-	-	-	-	5.309
			Biphasic	-	0.994	0.072	0.011	-	-	6.191
			Weibull	-	-	-	-	35.157	0.561	5.853
		50%	Linear	0.015	-	-	-	-	-	5.587
			Biphasic	-	0.969	0.086	0.009	-	-	6.379
			Weibull	-	-	-	-	12.536	0.357	6.648
		100%	Linear	-0.003	-	-	-	-	-	5.632
			Biphasic	-0.003	-	-	-	-	-	5.632
			Weibull	-0.003	-	-	-	-	-	5.632
	PVC	15%	Linear	0.013	-	-	-	-	-	5.921
			Biphasic	-	0.948	0.087	0.009	-	-	6.624
			Weibull	-	-	-	-	18.962	0.360	6.836
		50%	Linear	0.012	-	-	-	-	-	5.350
			Biphasic	-	0.989	0.406	0.008	-	-	6.812
			Weibull	-	-	-	-	10.383	0.303	6.456
		100%	Linear	-0.008	-	-	-	-	-	5.601
			Biphasic	-0.008	-	-	-	-	-	5.601
			Weibull	-0.008	-	-	-	-	-	5.601
	Tile	15%	Linear	0.010	-	-	-	-	-	5.682
			Biphasic	-	0.969	0.063	0.009	-	-	6.350
			Weibull	-	-	-	-	20.899	0.406	6.580
		50%	Linear	0.012	-	-	-	-	-	5.595
			Biphasic	-	0.944	0.571	0.010	-	-	6.587
			Weibull	-	-	-	-	19.754	0.340	6.472
		100%	Linear	-0.005	-	-	-	-	-	5.754
			Biphasic	-0.005	-	-	-	-	-	5.754
			Weibull	-0.005	-	-	-	-	-	5.754
7°C	SS	15%	Linear	0.010	-	-	-	-	-	5.702
			Biphasic	-	0.936	0.142	0.003	-	-	6.484
			Weibull	-	-	-	-	54.212	0.260	6.346
		50%	Linear	0.005	-	-	-	-	-	5.703
			Biphasic	-	0.921	0.054	0.001	-	-	6.238
			Weibull	-	-	-	-	75.500	0.237	6.367
		100%	Linear	0.006	-	-	-	-	-	6.165
			Biphasic	-	0.757	0.034	0.003	-	-	6.423
			Weibull	-	-	-	-	306.339	0.542	6.374
	PVC	15%	Linear	0.007	-	-	-	-	-	6.360
			Biphasic	-	0.850	0.058	0.004	-	-	6.788
			Weibull	-	-	-	-	92.329	0.371	6.878
		50%	Linear	0.005	-	-	-	-	-	6.237
			Biphasic	-	0.890	0.070	0.002	-	-	6.770
			Weibull	-	-	-	-	122.904	0.243	6.815
		100%	Linear	0.004	-	-	-	-	-	5.865
			Biphasic	-	0.974	0.081	0.000	-	-	6.793
e			Weibull	-	-	-	-	8.216	0.155	6.940
	Tile	15%	Linear	0.008	-	-	-	-	-	6.129
			Biphasic	-	0.917	0.047	0.004	-	-	6.602
			Weibull	-	-	-	-	56.669	0.362	6.762
		50%	Linear	0.004	-	-	-	-	-	6.020
			Biphasic	-	0.897	0.228	0.003	-	-	6.739
			Weibull	-	-	-	-	57.331	0.182	6.733
		100%	Linear	0.002	-	-	-	-	-	5.664
			Biphasic	-	0.939	0.047	0.000	-	-	6.371
			Weibull	-	-	-	-	195.730	0.199	6.325

Table 3. Goodness-of-fit values for the linear, biphasic, and Weibull models using GinaFit software for survival on different surfaces at different temperatures.

Temp	Surface	RH		R-square	R-square ADJ	MSE	RMSE
21°C	SS	15%	Linear	0.824	0.802	0.480	0.693
			Biphasic	0.983	0.975	0.060	0.246
			Weibull	0.954	0.941	0.144	0.379
		50%	Linear	0.811	0.787	0.284	0.533
			Biphasic	0.968	0.952	0.064	0.254
			Weibull	0.972	0.964	0.048	0.219
		100%	Linear	0.126	0.017	0.306	0.553
			Biphasic	-	-	-	-
			Weibull	-	-	-	-
	PVC	15%	Linear	0.808	0.784	0.222	0.471
			Biphasic	0.955	0.932	0.069	0.264
			Weibull	0.963	0.953	0.048	0.219
		50%	Linear	0.644	0.599	0.435	0.660
			Biphasic	0.954	0.931	0.075	0.274
			Weibull	0.947	0.931	0.074	0.273
		100%	Linear	0.343	0.261	0.752	0.867
			Biphasic	-	-	-	-
			Weibull	-	-	-	-
	Tile	15%	Linear	0.829	0.808	0.249	0.499
			Biphasic	0.976	0.963	0.048	0.218
			Weibull	0.971	0.963	0.049	0.220
		50%	Linear	0.780	0.752	0.228	0.477
			Biphasic	0.928	0.892	0.099	0.315
			Weibull	0.901	0.872	0.117	0.343
		100%	Linear	0.450	0.381	0.212	0.460
			Biphasic	-	-	-	-
			Weibull	-	-	-	-
7°C	SS	15%	Linear	0.596	0.546	0.161	0.401
			Biphasic	0.958	0.937	0.022	0.149
			Weibull	0.893	0.862	0.049	0.221
		50%	Linear	0.533	0.475	0.120	0.347
			Biphasic	0.981	0.972	0.006	0.080
			Weibull	0.918	0.894	0.024	0.156
		100%	Linear	0.629	0.583	0.106	0.325
			Biphasic	0.730	0.595	0.103	0.320
			Weibull	0.686	0.597	0.102	0.320
	PVC	15%	Linear	0.785	0.758	0.083	0.289
			Biphasic	0.953	0.930	0.024	0.155
			Weibull	0.957	0.945	0.019	0.138
		50%	Linear	0.538	0.480	0.103	0.321
			Biphasic	0.943	0.914	0.017	0.131
			Weibull	0.859	0.819	0.036	0.189
		100%	Linear	0.187	0.086	0.392	0.626
			Biphasic	0.879	0.819	0.078	0.279
			Weibull	0.688	0.599	0.172	0.414
	Tile	15%	Linear	0.770	0.741	0.121	0.347
			Biphasic	0.971	0.956	0.020	0.143
			Weibull	0.971	0.963	0.017	0.131
		50%	Linear	0.496	0.433	0.118	0.343
			Biphasic	0.908	0.861	0.029	0.170
			Weibull	0.924	0.902	0.020	0.143
		100%	Linear	0.070	-0.046	0.341	0.584
			Biphasic	0.683	0.525	0.155	0.393
			Weibull	0.374	0.195	0.262	0.512

Table 4. Regression parameters and goodness-of-fit data for the Baranyi and Roberts with no lag model using DMFit software for survival on stainless steel using different buffers.

Buffer	RH	R-Square	SE of Fit	Intial Value	Lag/Shoulder	Maximum Rate	Final Value
PEP	15%	0.909	0.47	5.959 ± 0.277	-	-0.0203 ± 0.00399	2.358 ± 0.273
	50%	0.809	0.505	5.998 ± 0.295	-	-0.0139 ± 0.00407	3.432 ± 0.298
	100%	-0.109	0.587	5.58 ± 0.325	-	0.00197 ± 0.00353	6.08 ± 0.421
PBS	15%	0.816	0.682	5.949 ± 0.467	-	-0.0358 ± 0.0119	2.402 ± 0.314
	50%	0.889	0.5	6.0849 ± 0.355	-	-0.0459 ± 0.0107	2.552 ± 0.223
	100%	-0.112	0.234	6.489 ± 0.0834	407.377 ± 721.368	0.00265 ± 0.0195	-
DW	15%	0.901	0.357	4.495 ± 0.19	-	-0.0312 ± 0.00582	1.672 ± 0.26
	50%	0.764	0.519	4.271 ± 0.318	-	-0.0497 ± 0.0166	1.944 ± 0.292
	100%	0.68	0.364	4.697 ± 0.32	-	-0.126 ± 0.0529	3.132 ± 0.148

Table 5. Regression parameters for the linear, biphasic, and Weibull models using GinaFit software for survival on stainless steel using different buffers.

Buffer	RH		kmax	f	kmax1	kmax2	delta	p	LOG10(N0)
PEP	15%	Linear	0.020	-	-	-	-	-	5.309
		Biphasic	-	0.994	0.072	0.011	-	-	6.191
		Weibull	-	-	-	-	35.157	0.561	5.853
	50%	Linear	0.015	-	-	-	-	-	5.587
		Biphasic	-	0.969	0.086	0.009	-	-	6.379
		Weibull	-	-	-	-	12.536	0.357	6.648
	100%	Linear	-0.003	-	-	-	-	-	5.632
		Biphasic	-0.003	-	-	-	-	-	5.632
		Weibull	-0.003	-	-	-	-	-	5.632
PBS	15%	Linear	0.019	-	-	-	-	-	4.889
		Biphasic	-	0.996	0.171	0.012	-	-	6.321
		Weibull	-	-	-	-	2.876	0.317	6.553
	50%	Linear	0.016	-	-	-	-	-	4.717
		Biphasic	-	0.999	0.110	0.010	-	-	6.120
		Weibull	-	-	-	-	28.516	0.490	5.513
	100%	Linear	0.000	-	-	-	-	-	6.500
		Biphasic	0.000	-	-	-	-	-	6.500
		Weibull	0.000	-	-	-	-	-	6.500
DW	15%	Linear	0.043	-	-	-	-	-	4.223
		Biphasic	-	0.961	0.115	0.025	-	-	4.657
		Weibull	-	-	-	-	9.854	0.450	5.009
	50%	Linear	0.036	-	-	-	-	-	3.716
		Biphasic	-	0.978	0.365	0.023	-	-	4.727
		Weibull	-	-	-	-	2.675	0.299	4.921
	100%	Linear	0.012	-	-	-	-	-	3.729
		Biphasic	-	0.975	0.361	0.000	-	-	4.740
		Weibull	-	-	-	-	0.912	0.116	4.830

Table 6. Goodness-of-fit values for the linear, biphasic, and Weibull models using GinaFit software for survival on stainless steel using different buffers.

Buffer	RH		R-square	R-square ADJ	MSE	RMSE
PEP	15%	Linear	0.824	0.802	0.480	0.693
		Biphasic	0.983	0.975	0.060	0.246
		Weibull	0.954	0.941	0.144	0.379
	50%	Linear	0.811	0.787	0.284	0.533
		Biphasic	0.968	0.952	0.064	0.254
		Weibull	0.972	0.964	0.048	0.219
	100%	Linear	0.126	0.017	0.306	0.553
		Biphasic	-	-	-	-
		Weibull	-	-	-	-
PBS	15%	Linear	0.727	0.693	0.776	0.881
		Biphasic	0.951	0.927	0.185	0.430
		Weibull	0.943	0.927	0.184	0.429
	50%	Linear	0.605	0.556	1.003	1.002
		Biphasic	0.945	0.917	0.187	0.433
		Weibull	0.799	0.742	0.584	0.764
	100%	Linear	0.006	-0.118	0.055	0.235
		Biphasic	-	-	-	-
		Weibull	-	-	-	-
DW	15%	Linear	0.849	0.831	0.219	0.468
		Biphasic	0.940	0.910	0.116	0.340
		Weibull	0.955	0.942	0.074	0.272
	50%	Linear	0.679	0.639	0.413	0.643
		Biphasic	0.889	0.834	0.190	0.436
		Weibull	0.908	0.881	0.136	0.369
	100%	Linear	0.217	0.119	0.365	0.604
		Biphasic	0.751	0.626	0.155	0.393
		Weibull	0.657	0.559	0.183	0.427

Table 7. Growth parameters and goodness-of-fit data for the Baranyi and Roberts model using DMFit software for growth using different buffers.

Buffer	R-Squared	SE of Fit	Initial Value	Lag/Shoulder	Maximum Rate	Final Value	Tmax
PEP	0.379	0.12	8.452 ± 0.116	-	0.0375 ± 0.0329	8.747 ± 0.0683	-
	0.962	0.117	6.761 ± 0.0832	-	0.0406 ± 0.00683	8.0886 ± 0.0837	-
	0.988	0.182	4.681 ± 0.169	4.804 ± 3.102	0.134 ± 0.02	8.195 ± 0.129	-
	0.984	0.296	2.505 ± 0.285	2.759 ± 2.566	0.217 ± 0.0317	7.497 ± 0.209	-
PBS	0.685	0.0671	8.478 ± 0.0481	-	0.0083 ± 0.00427	8.716 ± 0.0475	-
	0.688	0.0219	6.588 ± 0.0155	-	-0.0024 ± 0.00127	6.509 ± 0.0156	-
	0.954	0.101	4.492 ± 0.0743	9.607 ± 9.828	0.0183 ± 0.0031	-	-
	0.994	0.11	2.126 ± 0.0737	15.204 ± 3.609	0.0641 ± 0.0075	5.74 ± 0.379	-
DW	0.258	0.0867	8.398 ± 0.0474	31.193 ± 36.421	-0.0047 ± 0.0048	-	-
	0.473	0.067	6.293 ± 0.0622	0.0214 ± 0.0146	-	-	7.997 ± 4.53
	0.998	0.0194	4.582 ± 0.0114	20.962 ± 1.0735	0.0332 ± 0.00163	5.579 ± 0.0195	-
	0.992	0.0731	2.383 ± 0.0376	46.712 ± 4.347	0.0372 ± 0.00659	4.634 ± 0.0955	-

Figure 1. Survival of *E. aerogenes* on common surfaces stainless steel (A), PVC (B), and ceramic tile (C) at 21°C in desiccators containing salt solutions at 15 (●), 50 (▽), 100 (■)% RH. Error bars are expressed as ±SD.

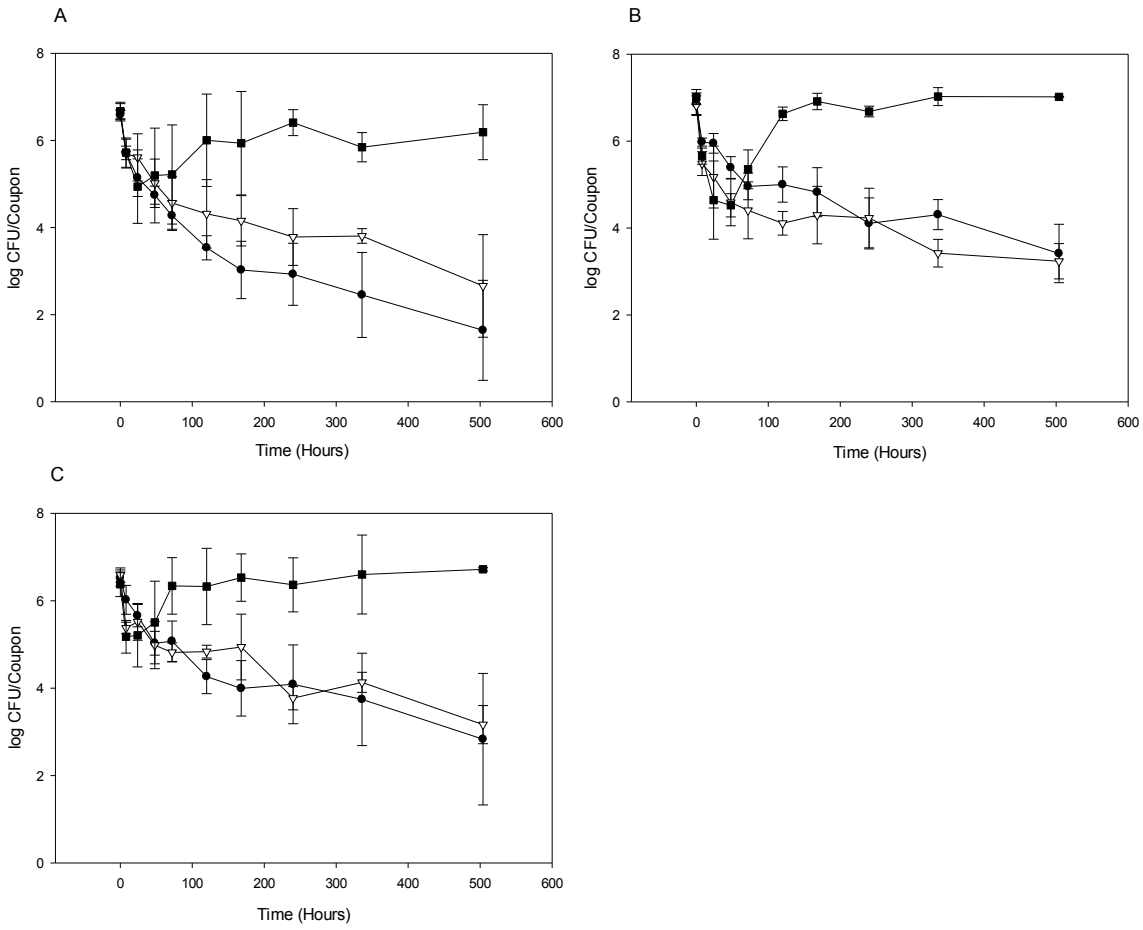

Figure 2. Survival of *E. aerogenes* on common surfaces stainless steel (A), PVC (B), and ceramic tile (C) at 7°C in desiccators containing salt solutions at 15 (●), 50 (▽), 100 (■)% RH. Error bars are expressed as ±SD.

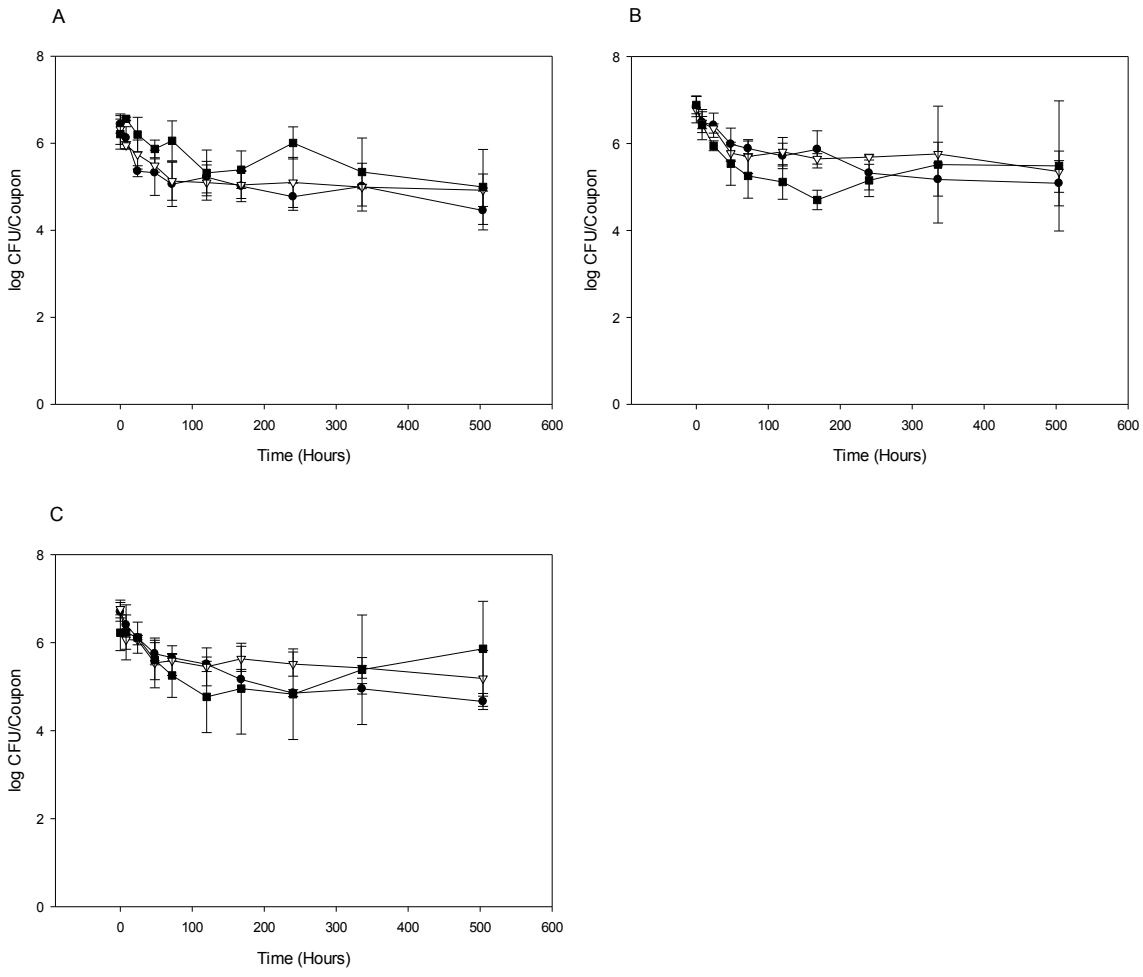

Figure 3. Survival of *E. aerogenes* on stainless steel using different buffers 0.1% peptone (A), 1% PBS (B), and distilled water (C) at 21°C in desiccators containing salt solutions at 15 (●), 50 (▽), 100 (■)% RH. Error bars are expressed as ±SD.

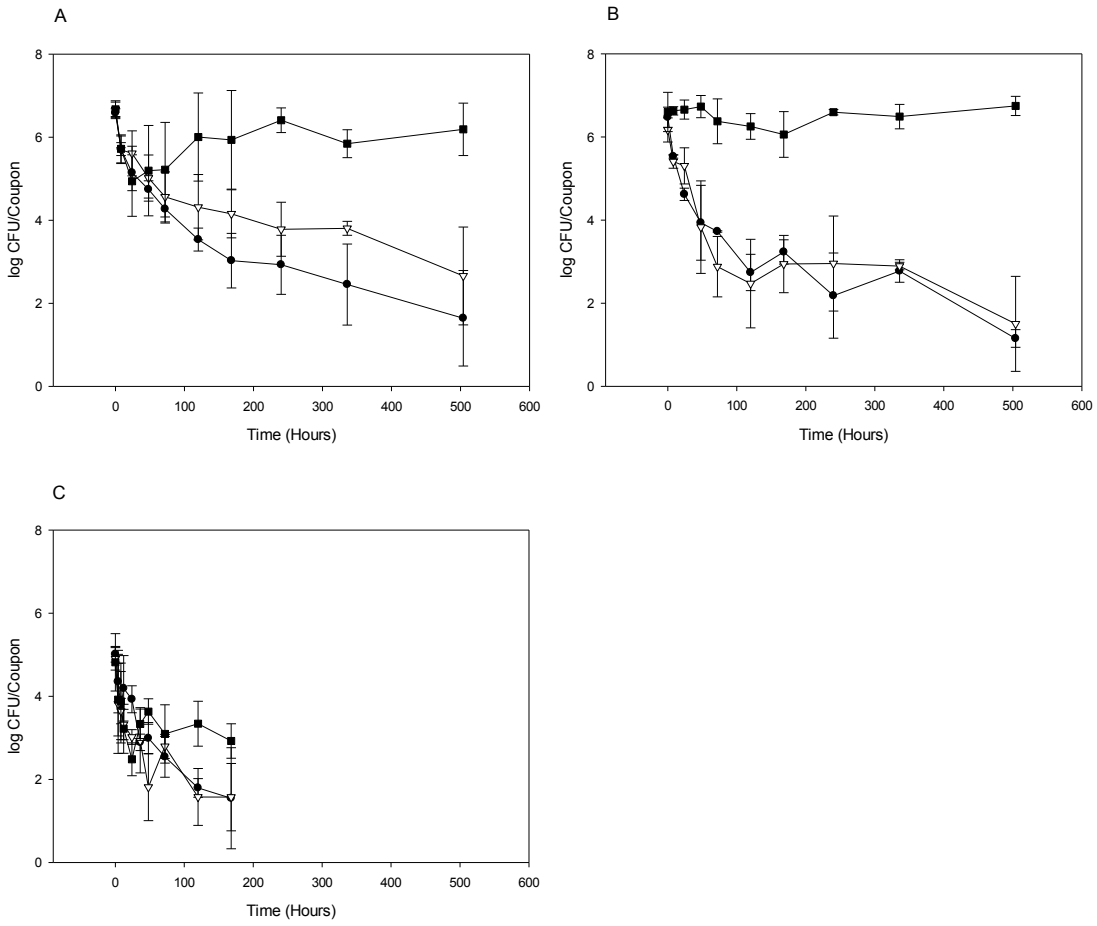

Figure 4. Growth of *E. aerogenes* using different buffers 0.1% peptone (A), 1% PBS (B), and distilled water (C) at 21°C at starting concentrations of ~2(□), 4(■), 6(▽), 8(●) log CFU/ml. Error bars are expressed as ±SD.

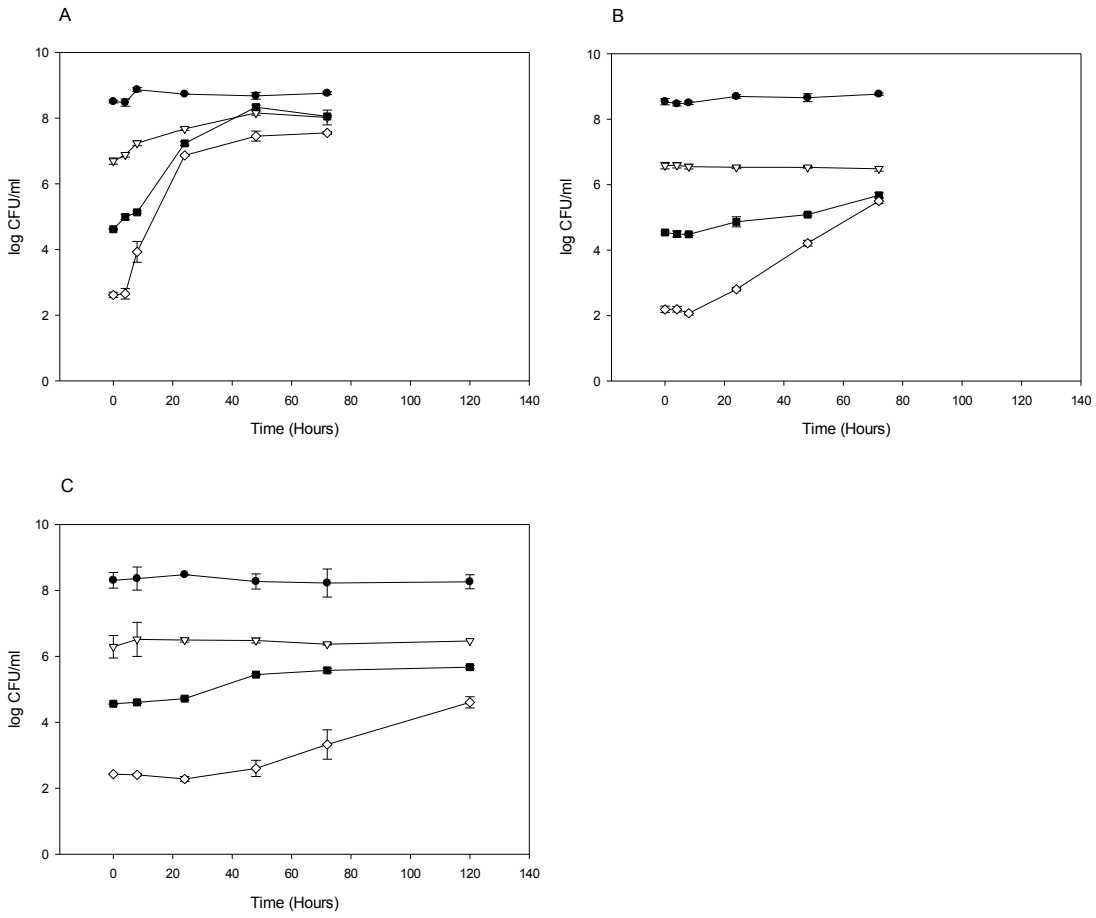

Figure 5. Growth of *E. aerogenes* on stainless steel using different buffers 0.1% peptone (A), 1% PBS (B) at 21°C in desiccators containing salt solutions at 100% RH at starting concentrations of ~2(■), 4(▽), 6(●), log CFU/coupon. Error bars are expressed as ±SD.