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The impact of various temperature and different concentration of salt on growth and survival of p. aeruginosa isolated from beef and macerated meat in the Karbala governorate

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***Abstract*— The purpose of this study was to examine how *P. aeruginosa* grew in beef and macerated meats at varying incubation temperatures and sodium chloride concentrations. Meat samples treated with 2%, 5%, 7%, and 10% sodium chloride were artificially contaminated using an inoculum containing 2.03 x 10^8 CFU/mL. Following 3 and 7 days of incubation at 7°C, 21°C, 37°C, and 45°C, growth was measured. Meat and Macerated meats only showed significant bacterial growth when exposed to 2% sodium chloride; in the Macerated samples, 5% sodium chloride showed very little bacterial growth. At salt concentrations of 7%, 10%, or more, no growth was seen, suggesting that high sodium chloride concentrations effectively inhibit *P. aeruginosa*.. With maximum growth at 37°C and restricted growth at 7°C and 21°C during early incubation, followed by total suppression after 7 days, temperature was found to have a substantial impact on bacterial survival. At 45°C, no bacterial growth was found. Higher bacterial counts were consistently found in Macerated meats, most likely as a result of increased surface area and nutrient exposure. These findings demonstrate that P. aeruginosa is sensitive to temperature and salt content, with higher tolerance seen in low-salt and moderate-temperature environments. The results highlight how crucial it is to regulate salt and temperature in order to lower the possibility of P. aeruginosa contaminating meat products.**

***Keywords* — Pesudomas aeruginosa, NaCl, Temperature, Inoculum .**

INTRODUCTION

THE *P. aeruginosa* is an opportunistic infection that frequently causes food that contains protein, especially meat and poultry products, to spoil (1). It has become a significant concern for food safety and preservation due to its capacity to grow in nutrient-poor circumstances and endure in humid situations. Often isolated from processed and refrigerated foods, this organism's proliferation causes discolouration, mucus formation, and unpleasant odors (2). Numerous studies have examined the effects of environmental variables, such as temperature and salt concentration, on *P. aeruginosa* growth, and the results have shown that ideal conditions are crucial for its spread.. According to reports, temperature has a significant impact on the rate and scope of bacterial development. It has been demonstrated that P. aeruginosa can survive at lower temperatures, albeit with reduced activity, despite being categorized as a mediterranean bacterium that grows best at about 37°C (3). On the other hand, heat stress-induced growth suppression has been linked to temperatures over 42 to 45°C. (4). Additionally, it has been determined that the concentration of sodium chloride (NaCl) has a significant impact on the metabolic and osmotic balance of bacteria. Higher quantities of NaCl have been shown to impede growth, and Pseudomonas spp. have been shown to be suppressed in meat systems at concentrations greater than 5% (5). Notwithstanding these established stress reactions, little is known about how temperature and NaCl solution interact to affect *P. aeruginosa* in different kinds of meat, especially in macerated tissues. In order to better understand *P. aeruginosa* behavior under typical food storage and processing conditions, (6).The objective of this study was to. examined the effects of varying sodium chloride concentrations and incubation temperatures on the organism's growth and survival in meat and pickled meat

MATERIALS AND METHODS

Samples collections

A total of 27 isolates from meat (10) and 17 from macerated meat samples to isolates *P. aeruginosa*. The samples were aseptically collected in 10 mL sterile plastic vials, with each sample obtained using sterile enrichment media to ensure proper preservation. The selection of samples was randomized to maintain unbiased representation,

Culture and identification

Using the procedure outlined by (6), the samples were streaked on nutrient agar plates and incubated for 24 hours at 37°C. Following Gram staining, suspected colonies exhibiting distinctive morphology were subcultured on selective and differential media, such as King's A agar, MacConkey agar, blood agar, and cetrimide agar. For additional examination, pure isolates of *P. aeruginosa* were moved to 1% nutritional agar slants and kept at 4°C. According to (7), biochemical testing, such as sugar fermentation assays, was used to validate the identification of *P. aeruginosa*.

Preparation of Inoculum *P. aeruginosa*

A loopful of *P. aeruginosa* were cultured in brain heart infusion broth, This broth was collected using phosphate-buffered saline (PBS, pH 7.2) after being incubated for eighteen hours at 37°C. After rinsing with PBS, the sample was centrifuged for five minutes at 7 °C at 2000 rpm, with the supernatant discarded. After that, the pellet was reconstituted in sterile PBS (pH 7.2). Viable plate count techniques were used to dilute and modify the suspension to a concentration of (1×10^{10} cells/mL) CFU/mL (8).

Enumeration of Bacteria

The experiment's bacterial count was calculated using several dilutions. A bacteria counting apparatus was used to count the bacteria in the samples after 100 microliters of bacteria were transferred to 99.9 milliliters of phosphate buffer saline, as seen in figure 1. Following a 24-hour incubation period at 37°C, the samples were plated on King A agar, and colonies that formed on the agar plates were counted (9).

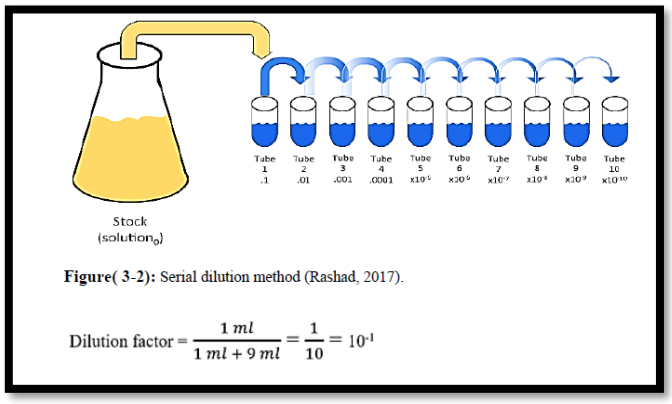

Figure 1. Serial dilution methods

- **Experiment consisted of two steps**

The experiment involved two steps

Temperature

P. aeruginosa was isolated from meat and macerated beef, and the bacteria were then cultivated on King A agar and incubated at the conditions listed below, 7°C to 3°C for 7 days, 21°C and 3 days, 37°C and 3 days, 45°C and 3 days**.**

Sodium chloride

As shown in the following tables, the bacteria were diluted in sodium chloride solutions that were made in accordance with the given concentrations. The bacteria were then cultivated on King A agar and incubated at the stated temperatures.

50 µl of an 18–24 hour broth culture was inoculated into 20 ml of the corresponding treatment tubes in order to evaluate the distinct impact of each salt concentration on *P. aeruginosa*. For the designated contact times, the infected tubes were vortexed and kept at room temperature (25ºC).. Viable counts were assessed using the spread plate method after 1 ml from each tube was serially diluted in 9 ml of sterile 0.1% peptone water at 1, 12, 24, and 96-hour intervals. Three duplicates of the experiment were carried out. The number of bacteria was expressed as CFU/ml or CFU/g. Only petri plates with *P. aeruginosa* colonies between 15 and 150 were taken into consideration for bacterial enumeration at the conclusion of the incubation period. Plates containing fewer than 15 colonies were designated "too few to count" (TFTC), while those with more than 150 colonies were deemed "too many to count" (TMTC) and disqualified from analysis A Quebec colony counter was used to count the colonies on each plate. The bacteria were cultivated on King A agar and incubated at the designated 2% sodium chloride as follows: 5% NaCl for 3 and 7 days, 7% NaCl for 3 and 7 days, and 10% NaCl for 3 and 7 days.

Meat contamination according to temperature

P. aeruginosa strain 106 was injected into macerated beef samples. For the inoculation, five grams or five milliliters of each sample were employed. Following that, the inoculation samples were incubated for three and seven days at temperatures of 4°C, 7°C, 21°C, 37°C, and 45°C under carefully monitored circumstances. Microbial analysis was performed to assess bacterial growth after incubation. To assess the bacterial concentration, each sample was serially diluted up to 10^6. To enable colony formation, aliquots from each dilution were plated on King A agar and incubated under the proper conditions.

To ascertain if the bacterial population had grown, shrunk, or stayed constant during the incubation period, colony-forming units were monitored and tallied. Each food product was evaluated for its suitability as a medium for *P. aeruginosa* growth under the testing conditions based on the presence or absence of colonies in the agar plates. To ensure the correctness of the observations and rule out outside contamination, infected control samples were kept in parallel.

Meat contamination according to salt concentration

Meat samples and macerated meat were infected with strain 106 to assess the impact of sodium chloride concentration on *P. aeruginosa* development. Different amounts of NaCl—specifically, 2%, 5%, 7%, and 10%—were added to each product. Five grams or five milliliters of each treated product were infected after the salt was added, and they were then incubated for three and seven days at 37°C. To assess the bacterial concentration, the samples were serially diluted up to a 10^6 dilution level after incubation. To enable colony formation, aliquots from every dilution were plated onto king A agar and incubated under the proper conditions to determine how salt concentration affected bacterial survival and growth, the number of colony-forming units was counted. To ascertain if increased salt concentrations impeded bacterial development or permitted persistence and adaptation, growth patterns were examined. The sterility of the goods was confirmed and the risk of external contamination was eliminated by maintaining infected control samples with equal salt levels

STATISTICAL ANALYSIS

Data were collected using a standardized form and tabulated in an MS Excel® spreadsheet. This program's data analysis tools were used to compile descriptive statistics. ANOVA was used to evaluate the data using SPSS version 23's General Linear Models technique (SPSS, 2019).

RESULT & DISCUSSION

Enumerate of bacterial count

A total of 203 colonies were obtained from serial tube number 6 form serial dilution of tubes and confirmed that used as inoculum strain figure 2

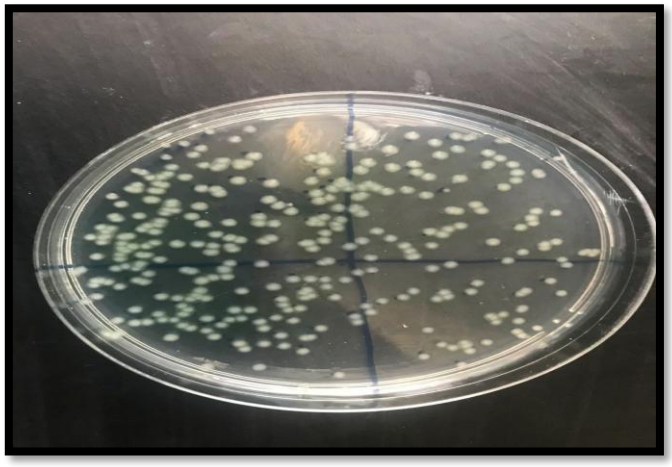

Figure 2. Inoculum strain of *Pseudomonas aerogenoza*

Meat contamination according to NaCl Concentration:

Over three and seven days at 37°C, the inoculum strain (2×10^8 CFU) was evaluated for its capacity to proliferate in meat and macerated meat at different concentrations of NaCl. Meat samples at 2% NaCl (68 CFU) showed strong growth after three days of incubation, but no growth was seen at higher concentrations (5%, 7%, and 10%; p = 0.0012). Macerated beef showed no growth at 7% and 10% NaCl (p = 0.021), minimal growth at 5% NaCl (5 CFU), and heavy growth at 2% NaCl (103 CFU). table 1 figure 3 A, Meat samples showed significant growth at 2% NaCl (180 CFU) following seven days of incubation, but no growth at 5%, 7%, or 10% NaCl (p = 0.0022). Likewise, macerated beef exhibited negligible growth at higher concentrations (p = 0.0012), with minimal growth at 5% NaCl (10 CFU) and considerable growth at 2% NaCl (215 CFU).table 1 figure 3 B.

Table 1. Growth of *P. aeruginosa* in meat and macerated meat under varying NaCl concentrations after 3 and 7 days of incubation at 37°C

Sample Type	NaCl (%)	3-Day Incubation (CFU)	7-Day Incubation (CFU)	P-value
Meat	2%	68 (Heavy growth)	180 (Heavy growth)	0.0012 (3-day)
	5%	0 (No growth)	0 (No growth)	0.0022 (7-day)
	7%	0 (No growth)	0 (No growth)	-
	10%	0 (No growth)	0 (No growth)	-
Macerated Meat	2%	103 (Heavy growth)	215 (Heavy growth)	0.021 (3-day)
	5%	5 (Minimal growth)	10 (Minimal growth)	0.0012 (7-day)
	7%	0 (No growth)	0 (No growth)	-
	10%	0 (No growth)	0 (No growth)	-

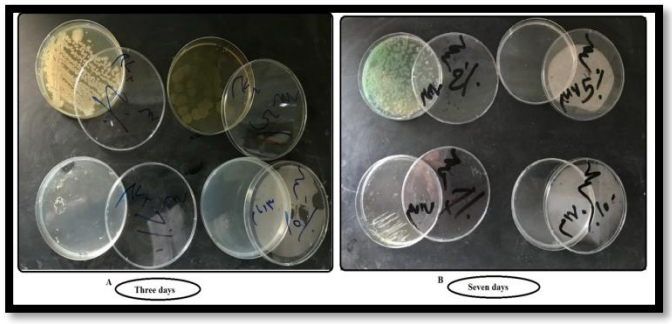

Figure 3. Growth of P. aeruginosa in meat in different concentration of NaCl

Meat contamination according to Temperature

After three days of incubation, the results showed that *P. aeruginosa* grew in both flesh and macerated meat samples at different temperatures. Meat (12 CFU) and macerated meat (17 CFU) showed limited growth at 7°C. At 21°C, there was an increase in growth, with 58 CFU in meat and 77 CFU in macerated meat. Both sample types showed significant growth at 37°C, but no growth at 45°C. With p-values of 0.0012 for beef and 0.0045 for macerated meat, statistical significance was verified (figure 4A)., However,

After seven days, neither the beef nor the macerated meat samples showed any signs of development at 7°C or 21°C. 180 CFU in meat and 155 CFU in macerated beef indicated heavy growth at 37°C, whereas 45°C showed no growth. Both beef and macerated meat showed statistically significant results (p = 0.0013 and p = 0.0024, respectively) (figure 4B).

Table 2. Growth characteristics of *P. aeruginosa* in meat and macerated meat samples at different temperatures during 3-day and 7-day incubation periods

Sample Type	Temp (°C)	3-Day Growth (CFU)	7-Day Growth (CFU)	P-value
Meat	7	12	0	0.0012 (3-day)
	21	58	0	0.0013 (7-day)
	37	Heavy	180	-
	45	0	0	-
Macerated Meat	7	17	0	0.0045 (3-day)
	21	77	0	0.0024 (7-day)
	37	Heavy	155	-
	45	0	0	-

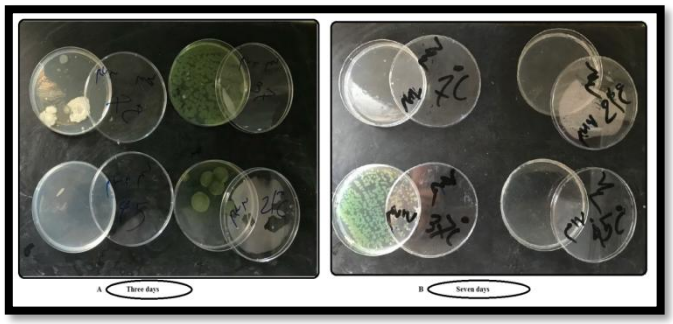

Figure 4. Growth of *P. aeruginosa* in meat under varying temperature after 3 and 7 days of incubation

DISCUSSION:

Similar to the approach used in this work, uniform colony counts obtained from repeated dilutions have been often used in prior research to prepare *P. aeruginosa* inoculum for food contamination models. According to reports, serial dilutions are commonly employed to accurately quantify the bacterial load before inoculating a variety of substrates, such as matrices made of meat, dairy, and vegetables. (9; 10). It is commonly acknowledged that the inoculum preparation of roughly 10^6 CFU/mL can replicate actual contamination scenarios, especially in perishable food goods where *P. aeruginosa* is known to thrive in the right conditions. Following this method, the inoculum employed in this study was obtained from the sixth dilution tube, producing 203 colonies, in line with procedures outlined in previous studies on microbial growth. Additionally, it has been shown that the physical and chemical characteristics of the medium can affect the viability and behavior of P. aeruginosa, which makes meat and macerated meat appropriate candidates for assessing bacterial adaptation and survival. (11, 12). The validity of standardizing bacterial burdens in foodborne contamination investigations by employing colony counts from serial dilutions is thus supported by the fact that the current inoculum preparation procedure may be regarded as being in line with previously accepted standards. In this investigation, pork and meat cooked with different concentrations of sodium chloride (2%, 5%, 7%, and 10%) were contaminated using an inoculum of P. aeruginosa (2.03 x 10^8 UFC/ml). After three and seven days of incubation, only a 2% sodium chloride concentration showed an increase in fungus, but no fungi were found at higher concentrations, suggesting that only 2% sodium chloride had a significant inhibitory effect. Although the reported UFC was significantly lower than 2%, which would have increased bacterial survival in mild water conditions, a slight increase in discolored meat was seen at a 5% sodium chloride concentration. These findings are in line with earlier research showing that P. aeruginosa is highly sensitive to concentrations over 4-5 percent, which may prevent it from engaging in metabolic processes and cellular functions. (2,13). Under circumstances of secondary bacteriuria, the incubation period may also promote bacterial proliferation, as seen by the notable difference in the rise between 3 and 7 days with 2% NaCl. It has been demonstrated that P. aeruginosa may flourish in low-salinity conditions and foods abundant in protein, like raw meat, but that high osmotic pressure significantly limits its ability to proliferate. (5). Additionally, the substantial rise in beef macerated with 2% to 5% NaCl could be explained by the higher bacterial colonization caused by the increased surface area and nutrition availability (11).

Therefore, it can be demonstrated that fresh meals offer a higher tolerance to moderate salt levels, and that sodium chloride concentration is a limiting factor in regulating *P. aeruginosa* in carnivorous items. Using an inoculum of 2.03 x 10^8 CFU/mL, the impact of incubation temperature on *P. aeruginosa* development in meat and macerated meats was assessed for 3 and 7 days. The organism clearly preferred temperate environments, as evidenced by the notable temperature-dependent variance in growth. Intense growth was observed at 37°C after three days, and after seven days, it spread to 180 CFU in beef and 155 CFU in macerated meats, indicating that this temperature is ideal for *P. aeruginosa* growth. However, no growth was seen in any sample type at 45°C, suggesting that heat stress at this high temperature prevents bacteria from surviving, as has previously been shown in other investigations.

(1,4). Both sample types showed considerable growth at 21°C after three days, but by day seven, the number of bacteria had dropped to zero, indicating that although moderate temperatures might enable short-term survival, they do not support long-term persistence. Limited growth was seen at 7°C after three days, but it vanished entirely

after seven, which is in line with studies that *P. aeruginosa* can endure for a short time under refrigeration but cannot sustain metabolic activity or replicate well at lower temperatures. *Pseudomonas spp*., (3). Greater nutrient availability and moisture retention, which have been demonstrated to support microbial adaptation under environmental stress, may be the cause of the more favorable growth outcomes seen in macerated meat as oppose to intact meat at suboptimal temperatures. (11). These results are consistent with the broader understanding that P. aeruginosa, although able to survive temporarily in cold environments, requires moderate to warm conditions to actively reproduce on protein-rich substrates (15).

CONCLUSION

Both the incubation temperature and the sodium chloride content had a significant impact on the growth of *P. aeruginosa* in cured meats. Only 2% sodium chloride showed significant bacterial growth, 5% sodium chloride showed only slight growth in cured meats, and greater salt concentrations showed no growth at all, suggesting that increasing osmotic pressure effectively inhibited the germs. It has also been demonstrated that temperature has a significant impact on the dynamics of bacterial growth and survival. During the early incubation periods, modest growth was noted at 21°C and 7°C, followed by total suppression by day 7. The optimal growth was noted at 37°C.

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