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Research Article

Influence of PH and Temperature on Growth Characteristics of Leading Foodborne Pathogens in a Laboratory Medium and Select Food Beverages

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Abstract

Accurate prediction of bacterial growth responsiveness over a range of processing environments is important for food processors to ensure proper food safety. Therefore, the aim of the current study was to evaluate growth characteristics of leading foodborne pathogens subjected to a range of pH levels (3 to 10) in a laboratory medium incubated at a range of temperatures (25 to 45°C). Bacterial lag times and growth rates were quantified at 620 nm for 48 hours via microtiter plate reader. This study was also validated by subjecting these pathogens into food beverages with different pH levels at 25°C. Results revealed that the densities of all species when subjected to pH 3 and 4 of laboratory medium at any tested temperature and all pH at 45°C, were limited to ≤ 3 log CFU/ml (inoculums level). The overall optimal levels for bacterial growth rate were pH 9 and 35°C. All species generally demonstrated lower growth rates in acidic environments than in alkaline environments. Data from studies with food beverages showed that all tested bacterial species with few exceptions were either maintained at inoculum level or increased no more than 1 log CFU/ml at pH ≤ 4.0, which further confirmed our laboratory medium findings. This study clearly demonstrates different responses by bacterial species to pH and temperature and will help inform decisions about the stringency of environments needed to reduce or control bacterial pathogen growth in foods.

Keywords: Lag time; Growth rate; Foodborne pathogens; pH; Incubation temperature

Introduction

Thorough understanding and adequate documentation of factors affecting the growth of foodborne pathogen is of great importance. Knowledge of bacterial growth responsiveness over a range of environments enables predictions of bacterial growth [1]. Using this information, questions about microbial food spoilage and food safety may be answered by objective analysis based on scientific data. This is especially relevant in light of the continuous occurrences of food product recalls and foodborne outbreaks throughout the world.

The generation time and lag phase of bacteria is greatly influenced by pH and temperature [2]. Consequently, food-manufacturing processes that modify either or both the pH and storage temperature of foods are extensively used as mechanisms for preventing microbial growth in foods and to ensure food safety [1]. Numerous studies have reported the approximate pH ranges [1,3-9] and temperature ranges [9-15] that limit growth of bacterial pathogens. Several scientists, however, reported tolerance and survival of foodborne pathogens in foods of non-optimal pH and temperature [16-20] and resistance to the lethal effects of very low pH [21]. Therefore, it is important, as addressed by Presser [1], to understand and be able to predict the responsiveness of microorganisms to pH and temperature changes. This information will assist in determining, as accurately as possible, the potential risks for contamination in different foods as well as the stringency of environments necessary to control their growth.

Most research documenting the pH and temperature tolerances of pathogenic bacteria has been conducted using a limited number of different bacterial strains or species in isolation. These studies are however difficult to compare due to differences in multiple variables, including strains or species of microorganisms, different research laboratories and environments and technologies used for the quantification of microbial growth. There may also be differences in methods used in assessing bacterial response to pH and incubation temperature. While little information is available in the literature, evaluating bacterial pathogen responses under concurrent environmental conditions will better elucidate the net effect of pH and temperature on their growth characteristics and thereby facilitate cross-species comparisons. Therefore, differences in bacterial lag time and growth rate as a function of pH and growth temperature were investigated to gain insight on the behavior of leading foodborne pathogens and to further document their growth characteristics.

Escherichia coli O157:H7, *Listeria monocytogenes, Salmonellaspp and Staphylococcus aureusremain* the leading bacteria accountable for the vast majority of foodborne illnesses, hospitalizations and deaths [22]. The advent of advanced technologies, eg. The microtiter plate reader enables rapid and simultaneous evaluation of the growth characteristics of microbes under any particular set of environments. In this first comprehensive study, we subjected these foodborne pathogens to a wide range of pH and different temperatures and evaluated the growth characteristics (lag times and growth rates)

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Table 1: Bacterial growth responsiveness in food beverages with different pH levels at 25°C after 48 h in cubation. Population of each bacterial species are presented in the unit of log CFU/ml.

Sample ID	pH	*B. cereus*	*E. coli* O157:H7	*L. monocytogenes*	*Salmonella* spp.	*S. aureus*
TSB+YE	7.3	> 6.0	> 6.0	> 6.0	> 6.0	> 6.0
Milk	7.0	5.8	> 6.0	> 6.0	> 6.0	> 6.0
Plain water	6.7	5.1	4.2	5.6	< 3.0	< 3.0
Coffee	5.6	< 3.0	< 3.0	< 3.0	4.3	< 3.0
Coconut water	5.5	5.1	> 6.0	> 6.0	> 6.0	> 6.0
Apple juice	4.0	< 3.0	< 3.0	< 3.0	3.5	< 3.0
V8 juice	4.0	3.0	4.0	3.3	> 6.0	> 6.0
Energy drink	3.7	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
Green tea	3.5	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
Gatorade	3.1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
Lemonade	3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
Coke	2.7	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0

using micro titer plates. A species of *Bacillus* cereus was also included, primarily to help understand the growth characteristic of toxin producers. We then validated our experimental results by inoculating these bacteria into food beverages with different pH and evaluating growth characteristics at room temperature (25°C).

Materials and Methods

Bacterial species used

Bacterial species used for the study were obtained from American Type Culture Collection (ATCC, Manassas, VA) and had been maintained in tryptic soy broth containing 20% glycerol at -80°C in our laboratory. Specifically, three strains of B. *cereus* (ATCC11778, 13061 and 14579), three strains of *E. coli* O157:H7 (ATCC 35150, 43888 and 700728), four strains of *L. monocytogenes* (ATCC 7644, 19115, 43256 and 51772), four strains of *S. aureus* (ATCC 6538, 29213, 33862 and 49444) and four strains of *S. enterica* (ATCC 13076, Enteritidis; ATCC 8387, Montevideo; ATCC 6962, Newport; and ATCC 1402 Typhimurimum) were used. Cultures were revived by transferring three times to tryptic soy broth (pH 7.3) supplemented with 0.6% yeast extract (TSBYE) by loop inoculation at successive 24h intervals and incubated at 37°C. Immediately before inoculation, a cocktail containing all strains (listed above) for each bacterial species was prepared by mixing approximately equal Colony Forming Units (CFU) of each strain and diluted to 105CFU/ml using sterile 0.85% saline solution. Appropriate dilutions of the homogenate were surface-plated on Standard Method Agar (SMA) for the quantification of their population after incubation at 36°C for 48 h. Each species of *B. cereus*, *E. coli* O157:H7, *L. monocytogenes, S. aureus and Salmonella spp*. was confirmed by plating on standard Mannitol Egg Yolk Polymyxin Agar (MYP), eosin Methylene Blue Agar (EMB), Modified Oxford Agar (MOX), Baird Parker agar supplemented with egg yolk telluride (BP) and Xylose Lysine Deoxycholate agar (XLD), respectively. Unless otherwise stated, all media were from Bacto (Becton Dickinson, Sparks, MD).

Procedure for bacterial growth

The pH of the growth medium (TSBYE) was adjusted using either 1N HCl or 1N NaOH from 3 to 10 at an interval of 1. Two hundred microtiter of each adjusted medium was dispensed into each well of sterile 96-well flat-bottomed microtiter plate (Costar 3595, Corning Inc., Corning, NY). The adjusted bacterial inoculum (105 CFU/ml) cocktail (2 µl) of each species were then distributed to each well in the micro titer plate containing the growth medium of TSBYE (200 µl/well). As a result, an inoculum concentration of approximately 1×10^3CFU/ml was obtained in each well. Optical Densities (OD) were measured after 5 sec auto mixing and recorded at 620 nm for each well at the start and every 20 minover a period of 48 hat temperatures of 25, 30, 35, 40 and 45°C using a multi-detection micro plate reader (Spectra Max 340PC, Molecular Devices, Sunnyvale, CA). In addition, the study was validated by inoculating the pathogens (3 logs CFU/ml) into commonly consumed food beverages (Table 1) with different pH levels, which were procured from a local retail market (Colonial Heights, VA). The beverages were incubated at 25°C for 48 h. Due to the difference of sample opaqueness; the validation study on food beverages was conducted by quantifying the level of viable bacteria in the well at the end of the incubation period. Bacterial suspensions in the wells were serially diluted, plated on SMA and counted. Representative colonies of each bacterial species on SMA were further confirmed on appropriate selective media.

Determination of lag time and growth rates

Turbid metric growth curves for each bacterial species were generated based on the changes in the OD due to bacterial growth over time. A representative example of the bacterial growth curves obtained from the laboratory medium on the micro plate reader is shown in (Figure 1). Lag time refers to the duration from inoculation to the onset of log-phase growth. Bacterial growth rates were computed with SoftMax® Pro Software and determined as the slope of the linear portion of the log-phase growth curve with $R2 \geq 0.92$ excluding the non-linear tails. While initial OD with inoculant level of 3 log CFU/ml ranged from 0.13 to 0.17, bacterial growth of OD less than 0.2 after 36 h of incubation were considered insignificant growth and excluded in the analysis.

Statistical analysis

The lag time and growth rate obtained for each bacterial species at the tested pH and temperatures from two replications of the

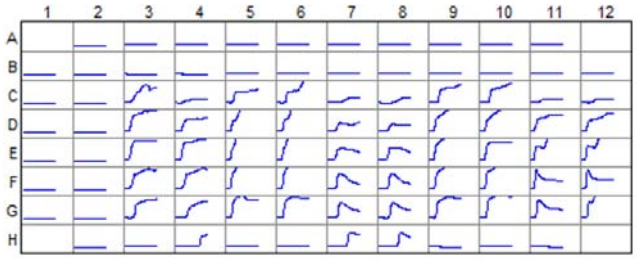

Figure 1: A representative growth curves for 5 different species of bacteria subjected to a range of pH levels (3 to 10) in a laboratory medium at 35°C. The horizon axes represent time in hours, while the vertical axes indicate Optical Density (OD) illustrating bacterial growth as measured at 620 nm.

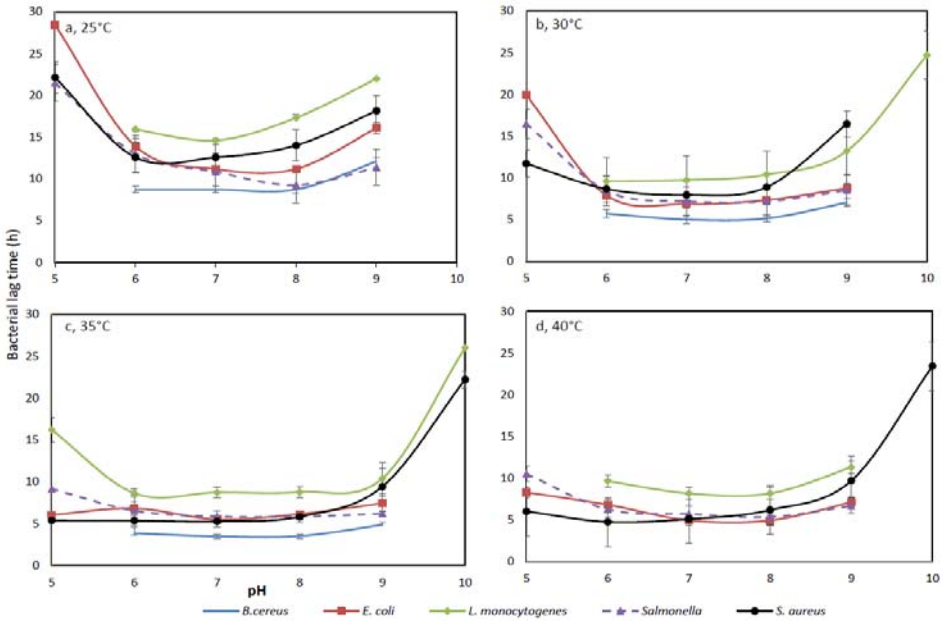

Figure 2(a-d): Effect of pH on the lag time of bacterial species in a laboratory medium at various temperatures. The standard error of the mean of each sample is shown in each Figure.

experiment were analyzed by one- and two-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (SAS Institute, Cary, NC, USA). Significance was defined at P<0.05.

Results and Discussion

Laboratory medium

Bacterial lag time: The results on the effect of pH (3-10)and incubation temperature (25 to 40°C) on the lag time of bacterial species in a laboratory medium (TSBYE) after 48 h incubation are shown in (Figures 2a-d). For all of the bacterial species tested, a clear pattern was noted where lag time decreased with an increase in pH from 5 to a pH of 6-8 depending on the species and then started increasing again from pH 9 (Figure 2a). In this study, we did not detect any growth at below pH 4 or pH 10 for the tested bacterial species. However, *E. coli*, *Salmonella* and *Staphylococcus* subjected to pH 5 were able to reach the onset of log-phase after 22 to 28 h incubation. In our study, observation of bacterial growth was conducted at pH intervals of 1. Therefore, the next level of pH where a change in OD was detected was taken as the onset of log-phase. For example, *Bacillus* had a lag time of 8.73hat pH 6 but no onset of log-phase was observed at pH 5, therefore, we considered *Bacillusdid* not grow at pH 5.

At pH 6, *Bacillus* showed the shortest lag time (8.73 h), while *Listeria* showed the longest lag time (15.93 h) indicating approximately one-half shorter time for *Bacillus* to reach the onset of log-phase. At pH 7 and 8, there was no significant difference (P>0.05) of lag time among *Bacillus, E. coli and Salmonella.* However, a significant (P<0.05) lag time difference between *Bacillus* and *Listeria* was observed with *Bacillus* showing the shortest lag time (8.73 h) at the pH range mentioned above while *Listeria* showed the longest lag times (14.60 h at pH 7 and 17.31 h at pH 8). The significant (P<0.05) difference of lag time between *Bacillus* and *Listeria* at 25°C incubation may be attributed to the combination of differences in bacterial size and optimum incubation temperature for each species. Holt [23] reported that the average size of *Listeria* is 0.4-0.5 µm in length by 0.5-2.0 µm in width, while Kaiser [24] described average size of *Bacillus* as 0.5-1.0 µm in length by 1.0-4.0 µm in width. Since the degree of turbidity in the microtiter plate, which spectrophotometer reads as OD, directly relates to how much light transmit bacterial suspension in TSBYE, *Bacillus* being almost twice the size of *Listeria* may have contributed to the detected shorter lag time than those in *Listeria* in addition to the increase of bacterial numbers along with incubation temperature. In other words, the population of *Listeria* in

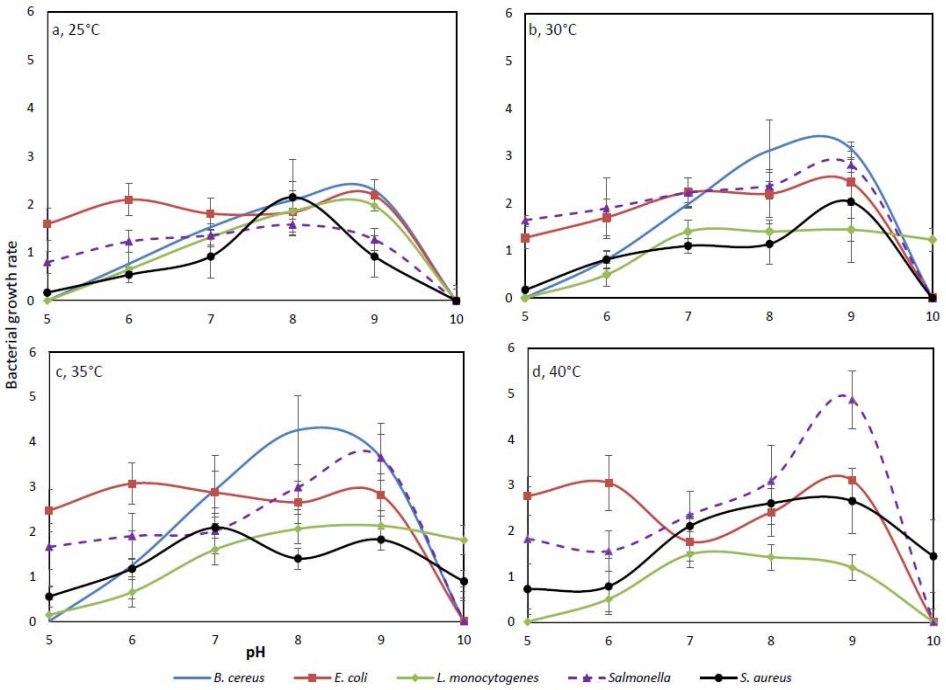

Figure 3a-d: Effect of pH on the growth rate of bacterial species in a laboratory medium at various temperatures. The standard error of the mean of each sample is shown in each Figure.

the suspension may need to be higher than those of *Bacillus* to reach the detectable level by the spectrophotometer.

According to the reports compiled by the New Zealand Ministry for Primary Industries [25,26], optimum temperature for the growth of *Listeria* is 37°C while *Bacillus* is 30 to 37°C. Therefore, the temperature (25°C) studied in (Figure 2a), which was closer to the optimum temperature for the growth of *Bacillus*, may have expedited the growth of *Bacillus* faster than *Listeria* resulting in shorter lag time of *Bacillus*. Although *Bacillus* and *Salmonella* showed the shortest lag time with no significant (P>0.05) difference at pH 9, *Salmonella* showed shorter lag time (11.37 h) than of *Bacillus* (12.09 h) indicating that *Salmonella* was less susceptible to alkaline pH than *Bacillus.*

When bacterial species were incubated at 30°C (Figure2b), similar patterns of the shortest lag time were observed at pH 7 in 25°C incubation (Figure 2a). The lag time at pH 7 among bacterial species was the lowest for *Bacillus* (5.00 h) and highest for *Listeria* (9.75 h) again indicating approximately one-half shorter time for *Bacillus* than *Listeria* to reach the onset of log-phase. The order was *Bacillus* (5.00 h), *E. coli* (6.87 h), *Salmonella* (7.19 h), *Staphylococcus* (7.96 h) and *Listeria* (9.75 h). There was no significant difference (P>0.05) of lag time among pH 6, 7 and 8 for all bacteria except *E. coli*, which had significantly (P<0.05) shorter lag time at pH 7. It is noted that *E. coli, Salmonella* and *Staphylococcus* subjected to pH 5 were able to reach the onset of log-phase after 20, 17 and 12 h incubation, respectively.

Results in (Figure 2b) revealed that at pH 6, there was no significant difference of lag time among bacterial species during incubation at 30°C. In addition, no significant difference (P>0.05) of lag time was observed among *Bacillus*, *E. coli* and *Salmonella* at pH 7, 8 and 9. *Bacillus* was the species that showed the shortest lag time (5.14 h at pH 8 and 7. 06 h at pH 9), while *Listeria* showed the longest lag time (10.40 h) at pH 8 and Staphylococcus (16.48 h) at pH 9, respectively. *Listeria* was able to reach the onset of log-phase after 25 h incubation at pH 10indicating that these bacteria may be least susceptible to extreme alkaline environment at 30°C incubation. FDA [9] and Lado and you [27] also reported that *Listeria* was able to grow at pH 9.6.

When bacterial species were incubated at 35°C (Figure 2c), all bacteria showed the shortest onset time of log-phase at pH 7 with no significant difference (P>0.05) among pH 6, 7 and 8. All bacterial species except *Bacillus* were able to reach the onset of log-phase at pH 5; Staphylococcus (5.37 h), *E. coli* (6.03 h), *Salmonella* (9.13 h) and *Listeria* (16.19 h). In addition, *Listeria* and *Staphylococcus* subjected to pH 10 were able to reach the onset of log-phase after 26 and 22 h incubation, respectively. No bacterial species grew below pH 4 and at pH 10 except *Listeria* and Staphylococcus when subjected to TSBYE at 35°C within 48 h. Results in (Figure 2) also revealed that at pH 6, 7, 8 and 9, *Bacillus* showed the shortest lag time while *Listeria* showed the longest lag time indicating approximately two to two and half shorter time for *Bacillus* than *Listeria* to reach the onset of log-phase. These results were consistent with the previous observations at 25 and 30°C incubations. It's interesting to note that it took longer time for *Salmonella* and *Listeria* to reach the onset of log phase at acidic environment (pH 5) than alkaline environment (pH 9). Both bacteria may be more susceptible to acidic pH than to alkaline pH. However, the opposite phenomenon was observed for Staphylococcus. The lag times at alkaline environments (pH 8 and 9) were longer than acidic environments (pH 5 and 6) indicating Staphylococcus may be more susceptible to alkaline pH than to acidic pH. When bacterial species were incubated at 40°C (Figure 2d), *E. coli* showed significantly

(P<0.05) shorter lag times at pH 7 and 8 than the ones at pH 5, 6 and 9. For *Listeria*, *Salmonella* and Staphylococcus, no significant difference (P>0.05) of lag times were observed among pH 6 to 8. It is noted that *E. coli*, Salmonella and Staphylococcus subjected to pH 5 were able to reach the onset of log-phase after a range of incubation times from 6 h to 11 h. In addition, Staphylococcus only was able to reach the onset of log-phase at pH 10 after 24 h incubation indicating that the microorganism may be least susceptible to extreme alkaline environment at 40°C incubation. Results in (Figure 2d) also revealed that *Bacillus* alone didn't grow at all tested pH. All bacterial species subjected to the tested pH ranging from 3 to 10 were unable to grow at 45°C incubation (data not shown).

The study revealed that the pH and temperature for the shortest onset time of log-phase of each bacterial species in a laboratory medium were as follows: *Bacillus*, 6 to 8 and 35°C; *E. coli*, 7 to 8 and 35 to 40°C; *Listeria*, 6 to 8 and 40°C; Salmonella, 8 and 35 to 40°C; and Staphylococcus, 6 to 8 and 35 to 40°C. The lag times were not significantly (P>0.05) different between pH levels and temperatures in those ranges. Overall, the optimum pH and incubation temperature obtained for the shortest lag time for *Bacillus*, *E. coli* and Staphylococcus was 7 and 35°C, while *Listeria* and Salmonella was pH 7 and 40°C and pH 8 and 35°C, respectively.

The lower and upper pH and temperature limit for growth found in the present study differ with the results compiled by Podolak [28] and Albrecht [29]. In their review, approximate pH and temperature values for the growth of each bacterial species were as follows: *Bacillus*, 4.9 to 9.3 and 4 to 48°C; *E. coli*, 3.6 to 10.0 and 4 to 45°C; *Listeria*, 4.5 to 9.6 and 1 to 45°C; *Salmonella*, 4.1 to 9.0 and 6 to 46°C; and *Staphylococcus*, 4.8 to 8.0 and 4 to 46°C indicating more acid and lower temperature tolerance than observed in the present study. Of the many potential variables, the detection method used in the present study may have played a key role in the interpretation of survival and growth of bacterial species in the tested pH and temperature ranges. As described in the materials and methods section, our method used OD more than 0.2 as an indication of bacterial survival, while in the other studies different recovery methods including broth enrichment and plating method in/on non-selective media were used. The latter methods may have made it possible to resuscitate injured cells due to pH and temperature shock resulting in different study findings. However, optimum pH and temperature obtained in our study were in agreement with their reports.

Bacterial growth rate. The effect of pH on the growth rate of bacterial species in TSBYE after 48 h incubation at a range of temperatures from 25 to 40°C is shown in (Figures 3a-d). As observed with lag time, growth rate pattern based on pH differences was evident at the different incubation temperatures. In general, optimal growth rates for all species were observed from pH 7 to 9 and at the different temperatures tested. When bacterial species were incubated at 25°C (Figure3a), the highest levels of growth rates were obtained at pH 9 and included; *Bacillus* (2.29), *E. coli* (2.19) and *Listeria* (1.97), while pH of 8was optimal for *Salmonella* (1.58) and *Staphylococcus* (2.15) indicating that the former three bacterial species may prefer more alkaline environments for their maximum growth than latter two species at this temperature. Although growth rates of *E. coli* and Salmonella varied, no significant (P>0.05) difference of growth rates due to pH from 5 to 9 was observed. At pH 7 and 9, the growth rate of Staphylococcus was reduced to approximately one-half of the growth rate seen at pH 8.In addition, *Bacillus* and *Listeria* were unable to grow at pH 5 indicating their less tolerance to acidic environment when incubated at 25°C.In general, *E. coli* was the least susceptible to acidic environment (pH 5).

We also found that at pH 6, *E. coli* showed a significantly (P<0.05) higher growth rate (2.10) than other bacterial species (Figure 3a). At this pH, growth rate of *E. coli* was almost three to four times faster than those of *Listeria* and Staphylococcus. Growth rates for the other tested species include; *Salmonella* (1.23), *Bacillus* (0.77), *Listeria* (0.65) and *Staphylococcus* (0.54). Interestingly, no significant (P>0.05) difference of growth rate was observed among bacterial species when subjected to pH 7 and 8. At pH 9, the growth rate of *Bacillus* was the highest (2.29) with Staphylococcus at the lowest (0.92) indicating *Bacillus* was the least susceptible and Staphylococcus the most susceptible to alkaline environment.

At 30°C, growth rates of all bacterial species were the highest at pH 9 (Figure 3b).When *Bacillus* was incubated at 30°C, the highest level of growth rate (3.15)was obtained at pH 9 while the lowest growth rate (0.81) was obtained at pH 6 (Figure 3b). However, no significant (P>0.05) difference of growth rates for all bacterial species except *Bacillus* were observed among pH from 6 to 9. While growth rates of *E. coli*, Salmonella and Staphylococcus were observed at pH 5, their rates were the lowest compared to other pH ranges. Similar to observations at 25°C, no growth of all bacterial species except *Listeria* at pH 10 was observed at pH 3, 4 and 10.

Our results showed that at pH 6, Salmonella (1.89) had the highest growth rate with *E. coli* (1.70), *Bacillus* (0.81), Staphylococcus (0.81) and *Listeria* (0.49) in descending order (Figure 3b). These results indicated that at pH 6, growth rates of *E. coli* and *Salmonella* were approximately three and half to four times faster than that of *Listeria*. At pH 7, the growth rates of *Bacillus*, *E. coli* and *Salmonella* were significantly (P<0.05) higher than those of *Listeria* and Staphylococcus. At pH 8, the growth rate of *Bacillus* (3.11) was the highest while the rate for other bacteria species were; *Salmonella* (2.37), *E. coli* (2.20), *Listeria* (1.40) and Staphylococcus (1.14). In general, *E. coli* and *Salmonella* were less susceptible than other bacterial species to acidic environments (pH 5 and 6) while *Bacillus* was the least susceptible to alkaline environments (pH 8 and 9).

When tested at 35°C (Figure 3c), *Bacillus* showed the highest level of growth rate (4.26) at pH 8. Although growth rate of *E. coli* varied from 2.47 to 3.07, there was no significant (P>0.05) difference among growth rates due to pH from 5 to 9. For *Listeria*, growth rates observed at pH from 7 to 10 were significantly (P<0.05) higher than those at pH 5 and 6 indicating *Listeria* was less active in acidic environment. The growth rate (3.65) of *Salmonella* was the highest at pH 9, while their growth rates were not significantly (P>0.05) different due to pH from 5 to 8. For Staphylococcus, growth rate (2.09) was the highest at pH 7. Significant (P<0.05) difference of Staphylococcus growth rate was observed between the pH range of 7-9 compared to the pH 5, 6 and 10 indicating Staphylococcus was more active in neutral to slightly alkaline pH environment (Figure 3c).

All bacterial species tested at 35°C were able to grow to some

extent at pH 5 except *Bacillus*, indicating *Bacillus* was less active in acidic environment than other bacterial species (Figure 3C). At pH 6, the growth rate of *E. coli* (3.07) was significantly (P<0.05) higher than other bacterial species. The growth rate for the other bacteria was; Salmonella (1.90), *Bacillus* (1.25), Staphylococcus (1.17) and *Listeria* (0.65). Growth rate of *E. coli* was approximately five times higher than *Listeria* at pH 6. We noted that at pH 7, growth rates of *Bacillus* (2.93) and *E. coli* (2.87) were significantly (P<0.05) higher than those of other bacterial species. The results obtained at pH 8 and 9 were similar to the previous findings in (Figure 3a,b) that growth rate of *Bacillus* was the highest and Staphylococcus the lowest. Although growth rates of *Listeria* and Staphylococcus were lower than other bacterial species at all tested pH, only these two bacteria were able to grow albeit slowly at pH 10 indicating these bacteria are least susceptible to extreme alkaline environment at 35°C.

At 40°C, *Bacillus* was unable to grow at all tested pH from 3 to 10. *E. coli* showed the highest growth rates at pH 6 (3.05) and 9 (3.10), which was significantly (P<0.05) higher than growth rate at pH 7 (1.76). The growth rate of *Listeria* was not significantly (P>0.05) different between pH of 7 to 9 but was significantly (P<0.05) higher than growth rate at pH 6. Moreover, the growth rates obtained at pH 9 were the highest for *E. coli*, Salmonella and Staphylococcus indicating that these bacteria are less susceptible to an alkaline environment when subjected to 40°C incubation. Similar growth phenomenon was also observed for all bacterial species except Staphylococcus at 25°C.

In this study, our results demonstrated that at pH 6, *E. coli* showed a significantly (P<0.05) higher growth rate (3.05) than other bacterial species (Figure 3d). Their growth rates recorded for the other bacteria in descending order were *Salmonella* (1.55), Staphylococcus (0.78) and *Listeria* (0.50). These results indicated that at pH 6, growth rate of *E. coli* was approximately four to six times faster than those of Staphylococcus and *Listeria*. No significant difference of growth rate was observed among bacterial species when subjected to pH 7. Moreover, at pH 8 and 9, the growth rate of *Salmonella* was the highest followed in descending order by *E. coli*, Staphylococcus and *Listeria*. In general, the growth rate of *E. coli* was the highest at acidic environments (pH 5 and 6) while the growth rate of *Salmonella* was the highest at alkaline environments (pH 8 and 9). No growth of bacteria was observed at pH 3, 4, 5 and 10 with the exception of growth of *E. coli*, Salmonella and Staphylococcus at pH 5. It is also noted that Staphylococcus was able to grow at pH 10.Regardless of the pH in the growth medium, all bacterial species tested here were unable to grow at 45°C (data not shown).

From the comparison of the growth rates of the five species at pH5, 6 and 7 and at all incubation temperatures ranging from 25 to 40°C, *E. coli* showed the highest growth rate. In addition, *Bacillus*, Salmonella and *Listeria* showed the highest growth rates at pH 8, 9 and 10, respectively. At 25°C incubation temperature, when all pH ranging from 5 to 10 were considered, *E. coli* showed the highest growth rate. When incubated at 30 and 35°C, *Bacillus* showed the highest growth rates while *Salmonella* showed the highest growth rate at 40°C.Specifically, the optimum pH and incubation temperature for growth of each bacterial species were as follows: *Bacillus*, 8 to 9 and 35°C; *E. coli*, 6, 7 and 9 and 35 to 40°C; Salmonella, 9 and 30 to 40°C; and Staphylococcus, 7 to 9 and 35 to 40°C, with no significant (P>0.05) difference in-between pH levels and temperatures tested. It was also noted that *Listeria* demonstrated a wide range of pH (7 to 10) and temperatures (25 to 40°C) for their optimal growth rate. Findings in this study clearly demonstrated that decreasing pH to ≤ 4 or increasing pH ≥ 10 with incubation temperature ≥ 45°Cin a growth medium can substantially decrease or halt growth of all bacterial species tested in the current study.

The present study using laboratory medium revealed that the pH and temperature range for the onset of log-phase growth of leading foodborne pathogens thus appear to be 6 to 9 and 25 to 40°C, with the optimum pH and temperature of 7 and 40°C, respectively. This agrees reasonably well with the results presented by others [10,25,26,28,29,35-38]. They reported that optimum pH and temperature values for the growth of *Bacillus*, *E. coli*, *Listeria*, Salmonella and Staphylococcus were 6.0 to 7.0 and 30 to 37°C, 6.0 to 7.0 and 37°C, 7.0 and 37°C, 7.0 to 7.5 and 35 to 43°C and 7.0 to 7.5 and 37°C, respectively. The optimum temperature found here (40°C) seems to be slightly higher than their result, which was approximately 37°C. The observed minor discrepancy in pH and temperature of bacterial growth between the present study and theirs may be attributed to the differences in multiple variables including strains or species, initial bacterial load, stage (log or stationary phase) of microorganisms and associated food matrices involved. A relatively larger temperature interval (5°C) assessed in the present study may have also partially attributed to the discrepancy.

Findings in this study revealed that although bacteria tested here are able to grow at a wide range of pH and incubation temperature, their lag time was the shortest at pH 7 and 40°C and their growth rate was the highest at pH 9 and 35°C. However, all species generally demonstrated lower growth rates in acidic environments than in alkaline environments. Based on the results, it appears that both pH and incubation temperature play a major role in the lag times and the growth rates of the microorganisms.

When all incubation temperatures ranging from 25 to 40°C were considered for mean values of growth rates and lag times, at pH 5, *E. coli* showed the highest growth rate than other bacterial species. At pH 6 and 7, *Bacillus* showed the shortest lag time while *E. coli* showed the highest growth rate. At pH 8, *Bacillus* showed the shortest lag time and the highest growth rate. At pH 9, *Bacillus* showed the shortest lag time while *Salmonella* showed the highest growth rate. When all pH ranging from 5 to 10 were considered, at 25°C incubation, *Bacillus* showed the shortest lag time while *E. coli* showed the highest growth rate. At 30 and 35°C, *Bacillus* showed the shortest lag time and the highest growth rate. At 40°C, *E. coli* showed the shortest lag time while *Salmonella* showed the highest growth rate. Discrepancies between the lag time and growth rate at log-phase in microbial species may indicate that microorganisms reaching the onset of log-phase growth fast does not necessarily reflect their speed of proportional reproducibility at log-phase.

In addition, our study revealed that increasing acidity (decreasing pH from 7 to 5) prolonged the onset of microorganisms to log-phase and decreased their growth rate significantly (P<0.05) while increasing temperature from 25 to 40°C in those pH ranges decreased lag time and increased growth rate significantly (P<0.05). Although increasing alkalinity (pH from 7 to 10) significantly (P<0.05) prolonged the onset

of microorganisms to log-phase, it did not significantly ($P>0.05$) affect bacterial growth rates. However, increasing temperature from 25 to 40°C in the pH ranging from 7 to 10 decreased lag time and increased growth rate of the microorganisms significantly ($P<0.05$).

Food beverages. Bacterial growth responsiveness in food beverages with different pH levels at 25°C during 48 h incubation are shown in (Table 1). Similar to our laboratory medium findings, most tested bacterial species with few exceptions were either maintained at inoculums level (3 logs CFU/ml), decreased or increased no more than 1 log CFU/ml in food beverages with pH ≤ 4. Results of growth of *E. coli*, *Salmonella* and *Staphylococcus* in V8 juice were consistent with the findings obtained in the laboratory medium, which showed that these species were more tolerant than other bacterial species to lower pH and were therefore able to grow in V8 juice with pH 4. Specifically, populations of *E. coli* inoculated into V8 juice were able to increase by approximately 1 log CFU/ml while both *Salmonella* and *Staphylococcus* exceeded 6 log CFU/ml. This study also noted that only *Salmonella* was able to increase by approximately 1 log CFU/ml in apple juice with pH 4 and coffee with pH 5.6. These observations were consistent with a FDA report [9] that the lower pH limit for the growth of *Salmonella* was 3.7. In the degree of acid tolerance of *E. coli*, our results differed from other reported studies and also showed inconsistency in growth between different food beverages with the same pH(V8 vs apple juice at pH 4).Studies done by Zhao and Doyle [17] and Baser [39] found that *E. coli* was not able to proliferate in foods with pH of less than 4 while others [1,40] reported that *E. coli* was able to grow even at pH 3.8. Nevertheless, some inconsistency in the growth characteristics of microorganisms at the same pH of different food beverages (V8 and apple juice) observed in our study may be due to the difference of preservatives that are normally added in the beverages for flavor and color enhancement. Interestingly, findings in our study revealed that even plain water with pH 6.7 could be a growth or sustainable medium for *E. coli*, *Salmonella* and *Bacillus* reaching their population up to 5.3 log CFU/ml within 2 days of incubation at 25°C. In addition, milk with pH 7.0 also proved to be a good medium recording bacterial growth to ≥ 6 log CFU/ml when left at room temperature (25°C) overnight.. Food products with this level of bacterial contamination can be potentially harmful if consumed. Therefore, findings here clearly demonstrate the need for careful safety practices in order to prevent any foodborne illness due to a low level of bacterial contamination in food products such as milk.

In summary, the knowledge obtained from the current research on the influence of pH and incubation temperature on the growth characteristic of leading foodborne pathogens in a laboratory medium and food beverages will significantly help understand bacterial responsiveness and further contribute to better control of those foodborne pathogens in the food industry. While much can be learned from the findings in this study, additional research efforts are needed to determine and validate the cause (s) of the observed discrepancy in bacterial growth associated with complicated food matrices (V8 and apple juice).

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