Development of an absorbance-based response model for monitoring the growth rates of *Arcobacter butzleri* as a function of temperature, pH, and NaCl concentration

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ABSTRACT In this study, the growth of *Arcobacter butzleri* in poultry was evaluated as a function of storage temperature (5, 22.5, and 40°C), pH (5, 7, and 9), and NaCl concentration (0, 4, and 8%). A predictive model was developed using the absorbance-based response surface methodology to describe the growth rate. The primary model was obtained to predict a growth rate with a good fit (R² ≥ 0.95), and the secondary model was obtained by nonlinear regression analysis and calculated as follows: Growth rate = −2.267274 − 0.024181 (Temp) + 0.6459384 (pH) + 0.1926227 (NaCl) + 0.0024661 (Temp × pH) − 0.001312 (Temp × NaCl) − 0.018802 (pH × NaCl) + 0.000467 (Temp²) − 0.041711 (pH²) − 0.007426 (NaCl²). Our data showed that the growth of *A. butzleri* can be completely inhibited at a pH of 5 (in the absence of NaCl, at 5°C) and at a pH of 9 (in the presence of 8% NaCl, at 5°C). The surface response model was statistically significant, with P < 0.0001, as evident from the Fisher F test and from coefficient determination (R², 0.95). This model was also verified by the bias factor (Bf, 0.839), accuracy factor (Af, 1.343), and mean square error (MSE, 0.0138). The newly developed secondary models of growth rate for *A. butzleri* could possibly be incorporated into a tertiary modeling program such as Pathogen Modeling Program (U.S. Department of Agriculture [USDA]) and Food Micro Model (in the United Kingdom). As a result, they could be used to predict the growth kinetics of *A. butzleri* as a function of a combination of environmental factors. Ultimately, the developed model can be used to reduce *A. butzleri* in poultry production, processing, and distribution, thereby enhancing food safety.

Key words: *Arcobacter butzleri*, predictive growth model, temperature, pH, NaCl

INTRODUCTION

*Arcobacter* species have been categorized as “aerotolerant Campylobacter” (Vandamme et al., 1991). They are widely distributed in water, animal, and foods of animal origin (Ho et al., 2006). *Arcobacter butzleri* is the most prevalent strain among *Arcobacter* species and is a motile, gram-negative, curved, s-shaped or helical non-spore-forming rod bacterium of the Campylobacteraceae family (Vandamme et al., 1991). *A. butzleri* is commonly isolated from diverse environments such as beef, pork, chicken (Phillips, 2001; Kabeya et al., 2004; Ho et al., 2008; Lee et al., 2010), lettuce (Gonzalez and Ferrus, 2011), reservoir and drainage water, milk, and pet animals, including cats and dogs (Scullion et al., 2006; Houtf et al., 2008; Pejchalova et al., 2008; Fera et al., 2009; Hausdorf et al., 2011). Specifically, *A. butzleri* was detected on 95% of chicken carcasses, 23% of frozen chicken carcasses (Atabay et al., 2003), 50% of turkey drumsticks, 57% of ground chicken, 86% of ground turkey, and 100% of whole frying chicken (Lammerding et al., 1996), indicating that poultry products are potential carriers.

*A. butzleri* have recently drawn attention because it causes foodborne outbreaks frequently, with the spread of enteritis and septicaemia (Kiehlauch et al., 1991; Vandamme et al., 1992; Lerner et al., 1994; On et al., 1995; Hsueh et al., 1997; Vandenberg et al., 2004; Shin et al., 2005; Jiang et al., 2010; Lappi et al., 2013). *A. butzleri* can grow aerobically and microaerobically at temperatures ranging from 15 to 37°C, with an optimum temperature of 30°C (Vandamme et al., 1991; Hilton et al., 2001). It can grow between pH 5 and approx. 8.5. Especially, it can grow well between pH 6 and 7 at 30°C (Hilton et al., 2001). In addition, the growth of *A. butzleri* was inhibited below water activity (A_w) 0.98, by using NaCl (Cervenka et al., 2003). Brain heart infusion agar in its basic formulation (5 g/L NaCl) is corresponded to A_w 0.995 (Cervenka et al., 2003).

There has been increasing interest in the development of mathematical models for the prediction of growth, survival, and inactivation of spoilage and pathogenic microorganisms in laboratory media and food matrices.
The mathematical models have been developed under diverse environmental conditions such as nutrient, oxygen, $A_w$, pH, temperature, and preservatives affecting bacterial growth, survival, and inactivation. Predictive food microbiology has now been established as an important microbiological and mathematical approach for ensuring food safety within the food supply chain (Koseki and Isobe, 2005).

Previously, quantitative mathematical models were used to improve the shelf life and safety of foods by adjusting environmental factors appropriately (Palumbo et al., 1991; Hudson, 1992; McClure et al., 1994; Gill et al., 1997; Sautour et al., 2003). Currently, there is only one useful and advanced model to predict the growth of *A. butzleri* under multiple combinations (12 to 37°C, pH 6.0 to 7.5, sodium chloride 0 to 3.5%, sodium nitrite 0 to 180 μg/mL, and sodium tripolyphosphate 0 to 0.012%), using Ellinghausen McCullough Johnson Harris (EMJH) medium (D’Sa, 2002). However, there is a need to further develop mathematical predictive models for the growth of *A. butzleri* under diverse stress conditions.

The aim of this study was to develop a growth predictive model for *A. butzleri* in broth, using three key environmental factors such as temperature, pH, and NaCl. The interactions between these factors have also been studied with the help of a response surface methodology (RSM).

**MATERIALS AND METHODS**

**Bacterial Culture**

*Arcobacter butzleri* ATCC 49616, isolated from human diarrheal stool samples collected in the United States, was purchased from American type culture collection (ATCC; Manassas, VA). ATCC 49616 is by far the most popular and widely used strain for the detection, isolation, and growth of *Arcobacter* spp. (Wesley et al., 1996; Rice et al., 1999; Fera et al., 2008). A stock culture on tryptic soy agar (TSA: Difco Laboratories, Detroit, MI) containing defibrinated sheep blood was maintained at −70°C in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) containing 50% glycerol. *A. butzleri* was cultured by transferring 10 μL of the stock culture into 10 mL of *Arcobacter* broth (Oxoid, Basingstoke, England), which was then kept at 37°C for 48 h in a gaseous atmosphere (78% N₂, 21% O₂, 0.9% Ar, 0.03% CO₂, and 0.07% vapor). The stock culture was subsequently harvested by centrifugation (8,000 × g rpm for 10 min at 4°C), washed with 0.1% peptone water (Oxoid, Basingstoke, Hampshire, UK) and diluted to 10² CFU/mL.

**Experimental Design for the Growth of Arcobacter butzleri in TSB**

Response surface methodology (RSM) is an empirical model tool used for a process improvement on bacterial growth. Central composite design (CCD) was used to predict the growth of *A. butzleri*, using software Design-Expert (Version 7.0, State-Ease Inc., Minneapolis, MN). Table 1 shows the CCD for a given range of parameters (based on coded and actual levels), incorporating the following three independent variables: incubation temperature, pH, and NaCl concentration. Low, intermediate, and high levels of each variable were designated as −1, 0, and +1 respectively. As a result, 20 combinations were chosen in random order according to a CCD configuration of the three factors (temperature, pH, and NaCl concentrations).

**Preparation and Inoculation of Culture Media**

Tryptic soy broth solutions containing 3 NaCl concentrations (0, 4, and 8%) were autoclaved at 121°C for 15 min and cooled. The pH of each NaCl solution was adjusted to 3 levels (5, 7, and 9 respectively), using 1 N NaOH or 1 N HCl and a glass electrode connected to a digital pH meter (Orion 3 Star, Thermo Electron Co., Waltham, MA, USA). Microplate wells were filled with 150 μL of the conditioned medium, to which 50 μL of the inoculum containing 10² CFU/mL of *A. butzleri* were added. Control wells containing 200 μL of uninoculated medium were used as blanks. Blank controls also confirmed the sterility of the medium. To confirm the sterility, the medium was incubated for several days, during which time no growth occurred.

**Table 1. Experimental design and results of the central composite design (CCD) in TSB for the primary modeling.**

<table>
<thead>
<tr>
<th>Run</th>
<th>$x_1$</th>
<th>$x_2$</th>
<th>$x_3$</th>
<th>Y (growth rate/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−1 (5)</td>
<td>−1 (5)</td>
<td>−1 (0)</td>
<td>−0.0600 ± 0.0864</td>
</tr>
<tr>
<td>2</td>
<td>−1 (5)</td>
<td>1 (9)</td>
<td>−1 (0)</td>
<td>0.1300 ± 0.0851</td>
</tr>
<tr>
<td>3</td>
<td>−1 (5)</td>
<td>−1 (5)</td>
<td>1 (8)</td>
<td>0.1200 ± 0.0500</td>
</tr>
<tr>
<td>4</td>
<td>−1 (5)</td>
<td>1 (9)</td>
<td>1 (8)</td>
<td>−0.1000 ± 0.0596</td>
</tr>
<tr>
<td>5</td>
<td>−1 (5)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.2000 ± 0.0187</td>
</tr>
<tr>
<td>6</td>
<td>0 (22.5)</td>
<td>−1 (5)</td>
<td>0 (4)</td>
<td>0.0340 ± 0.0288</td>
</tr>
<tr>
<td>7</td>
<td>0 (22.5)</td>
<td>1 (9)</td>
<td>0 (4)</td>
<td>0.2488 ± 0.0632</td>
</tr>
<tr>
<td>8</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>−1 (0)</td>
<td>0.3304 ± 0.0384</td>
</tr>
<tr>
<td>9</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>1 (8)</td>
<td>0.0484 ± 0.0596</td>
</tr>
<tr>
<td>10</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.2385 ± 0.0663</td>
</tr>
<tr>
<td>11</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.3708 ± 0.0404</td>
</tr>
<tr>
<td>12</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.2810 ± 0.0698</td>
</tr>
<tr>
<td>13</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.2382 ± 0.0663</td>
</tr>
<tr>
<td>14</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.2546 ± 0.0488</td>
</tr>
<tr>
<td>15</td>
<td>0 (22.5)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.3675 ± 0.0779</td>
</tr>
<tr>
<td>16</td>
<td>1 (40)</td>
<td>−1 (5)</td>
<td>−1 (0)</td>
<td>0.1167 ± 0.0046</td>
</tr>
<tr>
<td>17</td>
<td>1 (40)</td>
<td>1 (9)</td>
<td>−1 (0)</td>
<td>0.8436 ± 0.0308</td>
</tr>
<tr>
<td>18</td>
<td>1 (40)</td>
<td>−1 (5)</td>
<td>1 (8)</td>
<td>0.1210 ± 0.0641</td>
</tr>
<tr>
<td>19</td>
<td>1 (40)</td>
<td>1 (9)</td>
<td>1 (8)</td>
<td>0.0546 ± 0.0397</td>
</tr>
<tr>
<td>20</td>
<td>1 (40)</td>
<td>0 (7)</td>
<td>0 (4)</td>
<td>0.7025 ± 0.0273</td>
</tr>
</tbody>
</table>

$a_x$ = Temperature (5 to 40°C)  
$b_x$ = pH (5 to 9)  
$c_x$ = NaCl concentration (0 to 8%)
**Growth Temperature and Growth Measurement in TSB**

Three growth temperatures (5, 22.5, and 40°C) of *A. butzleri* were selected to represent practical food storage at refrigerator, room temperature, and elevated temperature, respectively. The growth rate of *A. butzleri* was monitored by measuring the optical density (OD) at 600 nm, using an automated microplate reader (ELx808, Biotech Ltd., Winooski, VT) and a microplate data-analysis software (Gen 5™, Biotech Ltd., Winooski, VT). All experiments were replicated 3 times. The absorbance values were natural log-transformed to maintain the homogeneity of variances. Absorbance measurements can be used as an alternative method for counting viable cells, which is time consuming and labor intensive. Absorbance measurements have generally been used to determine the growth rate (Dalgaard et al., 1994; Dalgaard et al., 1997; Neumeyer et al., 1997; Nerbrink et al., 1999; Zurera-Cosano et al., 2004; Park et al., 2007) during the exponential phase (owing to a high signal to noise ratio), although such measurements cannot effectively measure the slow growth that starts at the end of the initial lag phase (Nerbrink et al., 1999). In this study, we used absorbance as a measure of the growth rate and developed a predictive model for the growth of *A. butzleri* in TSB medium.

**Primary Modeling**

Growth data were iteratively fit to a modified Gompertz equation using a nonlinear regression model (Prism, Version 4.0, GraphPad Software, San Diego, CA) to determine the growth rate at each combination. The following (modified) Gompertz equation described by Gibson et al. (1988) was used for the above analysis:

\[
Y = N_0 + C \times \exp(\exp((2.718 \times \text{mue}/C) \\
\times \text{Lag} - X) + 1))
\]

Where:

Y = Log cell number at incubation time (X)
X = Incubation time
No = Log initial number of cells
C = Difference between initial and final cell numbers
mue = Growth rates
Lag = Lag time before growth, same units as X

For the curve-fitting of *A. butzleri* growth, we used the Gompertz equation, which was also used for fitting bacterial growth curves and estimating the lag times and growth rates by USDA (U.S. Department of Agriculture) researchers (Buchanan and Phillips, 1990; Palumbo et al., 1991; Buchanan et al., 1993; Bhaduri et al., 1994).

**Secondary Modeling**

In TSB, the growth rate for each environmental combination generated through CCD was determined by the least squares analysis of GLM procedure of SAS and fitted to the following quadratic polynomial model:

\[
Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_1X_3 \\
+ b_6X_2X_3 + b_7X_1^2 + b_8X_2^2 + b_9X_3^2 + \varepsilon
\]

Where:

Y = Growth rate
X1 = Incubation temperature
X2 = Initial pH
X3 = Concentration of NaCl
b0 = Intercept
b1, b2, and b3 = Linear coefficients
b4, b5, and b6 = Interaction coefficients
b7, b8, and b9 = Squared coefficients
\varepsilon = Random error

(Gibson et al., 1988) Statistical and adequacy testing of the model was performed using ANOVA. Statistical significance was determined based on an F value, with P > 0.05. Three-dimensional surface plots were constructed to determine the interactions of independent and dependent variables. The coefficient of determination (R^2) is often used as the overall measure of a prediction (Gibson et al., 1988). The R^2 values provided by GraphPad 4.0 are often used as an overall measure of the prediction attained. These values measure the portion of the variation about the mean, which can be explained by the model. The equation used to determine the R^2 value was as follows:

\[
R^2 = 1 - \frac{\sum e_i^2}{\sum (y_i - \bar{y})^2}
\]

Where:

e_i = Error of the predictive values
y_i = Predicted values
\bar{y} = Average of the predicted values

**Evaluation of Model Performance**

The mean square error (MSE), which is the residual sum of squares divided by the number of degrees of freedom, is a measure of the remaining variability that is not accounted for by deliberate changes in temperature. This value was calculated using the following equation: MSE = \[\Sigma \text{Log( observed values } - \text{ predicted values )}^2/n,\] where n is the number of observations.

The bias factor (Bf) answers the question of whether, on an average, the observed values lie above or below the line of equivalence and if so, by what extent. It also provides the structural deviations for the model. The equation used to calculate the bias factor was as follows:
RESULTS AND DISCUSSION

Predictive Response Surface Model of Arcobacter butzleri in a Tryptic Soy Broth–Based System

A primary model was developed based on the growth rate of *Arcobacter butzleri* at 20 different environmental conditions (temperature, pH, and NaCl) generated by a central composite design (CCD) of response surface methodology (RSM). Table 1 summarizes the growth rates of *A. butzleri* in tryptic soy broth (TSB) medium at 3 levels of pH (5, 7, and 9), NaCl (0, 4, and 8%) and temperatures (5, 22.5, and 40°C). The current study uses the Gompertz equation for curve-fitting the *A. butzleri* growth data. The Gompertz equation is typically used for fitting bacterial growth curves and for estimating the lag times and growth rates, by USDA (U.S. Department of Agriculture) researchers (Buchanan and Phillips, 1990; Palumbo et al., 1991; Buchanan et al., 1993; Bhaduri et al., 1994). Our data corresponding to the growth rates in TSB medium fitted well to the Gompertz equation model, exhibiting a very high degree of goodness of fits ($R^2 = 0.9500$ to 0.9995) for various combinations of pH, NaCl concentration, and temperature (data not shown).

The results obtained by CCD were analyzed by ANOVA (Table 1). The application of multiple regression analysis was related to the following polynomial equation:

$$Y = -2.267274 - 0.024181\text{Temp} + 0.6459384\text{pH} + 0.1926227\text{NaCl} + 0.0024661\text{Temp} \times \text{pH} - 0.001312\text{Temp} \times \text{NaCl} - 0.018802\text{pH} \times \text{NaCl} + 0.000467\text{Temp}^2 - 0.041711\text{pH}^2 - 0.007426\text{NaCl}^2$$

The statistical significance of the model was determined using the Fisher $F$ test (Yu et al., 2008) and coefficient determination ($R^2$). As demonstrated previously by other groups (Duffy et al., 1994; Grau and Vanderlinde, 1993; Sutherland et al., 1994), higher values of $R^2$ (0 < $R^2$ < 1) indicate that predictions made by the model are of sufficiently good quality. Our model was highly reliable, as evident from the Fisher $F$ test ($F = 15.86$) and from the low $P$-value ($P < 0.0001$) (Table 2). Finally, the goodness of the regression was also validated with an $R^2$ value of 0.95 (Table 2). Previously, $R^2$ value in a response surface model of *A. butzleri* in Ellinghausen McCullough Johnson Harris (EMJH) medium was reported to be less than 0.90 when temperature, pH, NaCl, and sodium tripolyphosphate (STPP) were used (D’Sa, 2002).

### Predictive Growth Rates of A. butzleri at Various Combinations of Temperature, pH, and NaCl Concentrations in a TSB-Based System

The polynomial equation estimated the predicted growth rates for *A. butzleri*, at various combinations of temperature, pH, and NaCl concentration in TSB medium (Table 1). For example, the predicted growth of *A. butzleri* was completely inhibited at 5°C, pH 5, and 0% NaCl as well as at 5°C, pH 9, and 8% NaCl. The growth rates of *A. butzleri* were negative under these conditions, thereby indicating growth inhibition. At a storage temperature of 22.5°C, there were 4.29-fold decreases in the growth rates in the combination of pH 7 and NaCl 8% (0.0679 h⁻¹) compared to the combination of pH 7 and NaCl 0% (0.2912 h⁻¹). At a storage temperature of 22.5°C, 0.2984 h⁻¹ of *A. butzleri* was observed in the combination of pH 7 and NaCl 4%. Our results were somewhat similar to those obtained by D’Sa (2002), who reported a growth rate of 0.34 h⁻¹ for *A. butzleri* ($t = 24.5°C$, $pH = 6.75$, NaCl = 1.75%, and STPP = 0.006%). At a storage temperature of 40°C, the growth rate for *A. butzleri* achieved a maximum (0.8353 h⁻¹), in the absence of NaCl and at a pH of 9. This result was in moderate agreement with a previous study by Hilton et al. (2001), who reported that the maximum growth of *A. butzleri* NCTC 12481 was observed between pH 5.0 and 8.5, when the temperature was 30°C. In general, it has been observed that *A. butzleri* can grow in air at 30°C, after primary isolation in microaerobic conditions (Vandamme et al., 1991).

Figure 1 shows the effect of independent variables (temperature, pH, and NaCl concentration) on the predicted growth rate of *A. butzleri*, as indicated by 3-dimensional response surface plots. Figure 1 shows the response surface plot for the growth of *A. butzleri* as a function of temperature and pH, in the complete absence of NaCl. The growth rate saw an increase at higher temperatures and at higher pH values. However,

### Table 2. Statistical indices of the secondary response surface modeling step for the growth rate of A. butzleri in TSB.

<table>
<thead>
<tr>
<th>Model</th>
<th>$P^*$</th>
<th>$R^2$</th>
<th>$B_f$</th>
<th>$A_f$</th>
<th>MSE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response surface model</td>
<td>$&lt; 0.001$</td>
<td>0.950</td>
<td>0.839</td>
<td>1.343</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

* $P$ = Probability
* $R^2$ = Coefficient of determination
* $B_f$ = Bias factor
* $A_f$ = Accuracy factor
* MSE = Mean square error
Figure 1. Response surface model to predict growth rates of *A. butzleri* in combination of temperature and pH in TSB.

Figure 2. Response surface model to predict growth rates of *A. butzleri* in combination of pH and NaCl in TSB.

growth rates tended to decrease when the pH was close to 9. Figure 2 shows the response surface plot for the growth of *A. butzleri* as a function of pH and NaCl concentrations at 37°C. The growth rate was generally decreased at pH 9 and 5. The growth rates tended to increase until the NaCl concentration reached 4%. The growth rates were affected more by the NaCl concentrations than by pH values. Figure 3 shows the response surface plot for the growth of *A. butzleri* as a function of temperature and NaCl concentration, at a pH of 7. The growth rate was generally decreased at lower temperatures and at a higher concentration of NaCl. However, the growth rate at 22.5°C was higher at 4% NaCl and lower at 0% NaCl.

*A. butzleri* can grow in a liquid medium at 10°C, after an incubation period ≥7 d (D’Sa and Harrison, 2005), and in water at 4 and 7°C, before the number of cells eventually starts decreasing (Van Driessche and Houf, 2008). In a study conducted by D’sa and Harrison (2005), *Arcobacter* spp. exhibited growth in up to 3.5% NaCl, depending on the species and strain under investigation. Some strains survived at NaCl concentrations up to 5.0%, which corresponded to water activity (A_w) of approximately 0.968, especially at nonoptimal growth temperatures. In addition, *A. butzleri* growth was inhibited below A_w 0.980, by adjusting the concentration of NaCl (Cervenka et al., 2003).

Validation of Predictive Response Surface Model

The reliability of RSM based on goodness of fit, requires mathematical evaluation before practical application. Figure 4 shows the comparative plots based on the actual and predicted growth rates of *A. butzleri*. Plotted points were generally close to the equivalence line, indicating satisfactory performance of the predictive model. Scatter plots of the observed versus predicted data were also used to assess the success of the model predictions. Most of the points were relatively close to the line corresponding to a 100% correlation (y = x), thereby indicating satisfactory performance of the predictive model (Eifert et al., 1996; Fernandez et al., 1997).

Figure 3. Response surface model to predict growth rates of *A. butzleri* in combination of temperature and NaCl in TSB.

Figure 4. Comparison of the observed and predicted growth rates for *A. butzleri* in TSB.
The bias factor (B₁), accuracy factor (A₁), and mean square error (MSE) were calculated to evaluate the predictive models. A B₁ < 1 indicates a “fail-safe” model, and a B₁ > 1 indicates a “fail-dangerous” model (Ross, 1996). According to (Ross, 1996; Park et al., 2007; Seo et al., 2008), B₁ value from 0.9 to 1.05 should be considered good, with the values of 0.7 to 0.9 or 1.06 to 1.15 for acceptable and the values < 0.7 or > 1.5 for unacceptable. The B₁ value in our model was 0.839, which was in the acceptable range. The B₁ value does not provide an indication of the average accuracy of estimates, so the A₁ value was also calculated (Dong et al., 2007). As the value of A₁ increases, the accuracy of the model decreases. An A₁ value in the range 1.3 to 1.5 can be considered to be good (Sutherland et al., 1994; Park et al., 2005; Jin et al., 2006; Park et al., 2007). Our average estimate of the A₁ value was 1.343, which was within the acceptable range. The lower the MSE value, the better the adequacy of the model (Adair et al., 1989; Sutherland et al., 1994). The MSE value for our model was 0.0138.

The developed response surface model proved reliable for predictions of the combined effects of temperature, pH, and NaCl concentration on the growth rate for A. butzleri in TSB. It is possible to minimize the risk of food poisoning by adjusting the environmental conditions based on the growth rates. However, it is not easy to guarantee the complete elimination of a pathogen in all environments. The model developed in this study provides a useful tool to determine the probability of A. butzleri growth in certain conditions during food production, processing, and distribution. However, for risk management, further work is necessary to confirm the prediction of the growth rate model for A. butzleri in food products.

Conclusions

This experiment verified that A. butzleri is highly sensitive to adverse growth conditions. The organism cannot vigorously grow when exposed to low refrigeration temperatures, acidic conditions, and/or high NaCl concentrations. The predictive model developed here was in good agreement with the observed values, which indicates that it could be used as a practical model to predict the growth of A. butzleri. There is currently an urgent need to develop models for predicting the growth, death, and transmission of A. butzleri in diverse food matrices and food processing facilities, under various environmental conditions. Therefore, the newly developed secondary models of growth rates for A. butzleri in a TSB-based system could possibly be incorporated into a tertiary modeling program such as Pathogen Modeling Program (USDA) and Food Micro Model (in the United Kingdom), such that they could easily be used to predict the growth kinetics of A. butzleri as a function of a combination of environmental factors. Ultimately, the developed model is vital in reducing the levels of A. butzleri in poultry production, processing, and distribution, thereby enhancing food safety.

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REFERENCES


