AN EMPIRICAL MODEL TO PREDICT ENTEROTOXIN A PRODUCTION BY *STAPHYLOCOCCUS AUREUS*

W.S. Robazza¹, G. Fraga¹, V.B. Souza¹, A.C. Galvão¹

1-Food and Chemical Engineering Department – Santa Catarina State University, Av. Coronel Ibiapina de Lima, 750, Centro – CEP: 89870-000 – Pinhalzinho – SC – Brazil, Telephone: 55 (49) 2049-9598 – Fax: 55 (49) 2049-9593 – e-mail: (wrobazzi@yahoo.com.br; gabrielafraga@gmail.com; valbarreira@gmail.com; eng.a.c.galvao@gmail.com)

ABSTRACT – A mathematical model to predict the production of Enterotoxin A by *Staphylococcus aureus* was developed and analyzed. The model includes two rate constants: one that describes a linear stage and other that describes an exponential stage of the production of toxins that occurs during the stationary phase of bacterial growth. The model was fitted to four published data sets obtained for different products subjected to different conditions of storage. Results showed a good agreement between the experimental data and the model which indicates that such equation may provide useful information for risk assessment studies.

KEYWORDS: *Staphylococcus aureus*; Enterotoxin A; mathematical model.

1. INTRODUCTION

*Staphylococcus aureus* is a pathogen of great concern to the food industry. According to the Centers for Disease Control and Prevention (CDC), it is responsible for about 240,000 illnesses with 1,000 hospitalizations and 6 deaths associated with staphylococcal poisoning annually (Scalan et al., 2011). The contamination of food products with *S. aureus* may result from the lack of hygienic practices in food processing environment (Gutiérrez et al., 2012). The high incidence of outbreaks due to *S. aureus* can be explained by the ubiquitous presence of these bacteria on nasal cavity and skin surfaces (Alves et al., 2014). Staphylococcal poisoning is directly linked to the ability of certain strains to produce heat stable, tolerable low pH enterotoxins (Tallent et al., 2013).

Staphylococcal enterotoxins (SEs) are a group of single chain with low molecular weight compounds (27,000-34,000) that are produced during all phases of bacterial growth, but mainly during the middle and at the end of the exponential phase (Soriano et al., 2012; Rosengren et al., 2013). To date, 23 serologically different SEs have been identified and classified (Tango et al., 2015). The amount of enterotoxin required to produce illness depends on the susceptibility of the individual to the toxin, weight and the overall health of the affected person. The minimal dose of the main serotype, Staphylococcal enterotoxin A, (SEA) reported to cause disease in children school was 144±50 ng (Evenson et al., 1988).

As a guideline, it is required $10^5$ staphylococci colony-forming units (CFU/g) to produce sufficient enterotoxins to cause illness (US Food and Drug Administration, 1992). This value is in good accordance with results observed in outbreaks that occurred due to contamination of different food products (Fujikawa and Morozumi, 2006; Tango et al., 2015). Therefore, any mathematical model designed to predict the production of SEs, should include a critical microbial population required to produce SEs as a key parameter. In addition, it must evaluate microbial growth as well enterotoxin production.

Predictive microbiology is an area of food microbiology which has come to be known in the last twenty five years. It consists in a quantitative science that enables users to evaluate objectively the effect of processing, distribution, and storage operations on the microbiological safety and quality of foods (McMeekin et al., 1993). The models found more often in the field are the modified Gompertz
model (Gibson and Roberts, 1986) and the Baranyi model (Baranyi and Roberts, 1994). However, Baranyi growth model is the most commonly used equation because it provides a mechanistic basis for the duration of the lag phase (Baranyi and Roberts, 1995).

The main objective of this study was to predict and evaluate a mathematical model that predicts the production of SEA in different food products. In order to evaluate microbial growth, it was used the Baranyi model and a new equation was proposed to evaluate the enterotoxin production. To test the ability of the model to deal with experimental data, four published data sets were selected and analyzed. The parameters obtained were used to estimate the minimal microbial population required to produce SEAs and a rate constant of the reaction of production of the toxins.

2. MATERIALS AND METHODS

2.1 Model Description

Throughout this study, in order to describe the growth of S. aureus, it was employed the Baranyi growth model (Barany and Roberts, 1994). The model is complicated, but there are softwares, such as R, the open source statistical software (R Core Team, 2013) that facilitates its practical use for curve fitting. Baranyi’s growth model was used to evaluate the log count of S. aureus as a function of time. This result was used as an input in the SEA production model, which is described below.

As an underlying hypothesis of the SEA production model, it was assumed that the rate of production of SEA is directly proportional to the difference of the microbial log count and the minimal log count required to the bacteria produce SEA. In other words, this means that, the higher the microbial population, the higher will be the concentration of SEA produced. These hypotheses can be described by a single differential equation, expressed by Equation 1:

$$ \frac{dSEA(y)}{dy} = k(y - y_c) e^{\beta t} $$

where $y$ is the microbial log count given by Baranyi growth model, $y_c$ is the minimal microbial population required to SEA production, SEA stands for the concentration of enterotoxin A, $\beta$ is a rate constant related to the production of enterotoxin during the stationary phase of growth, $t$ stands for the time and $k$ is a rate constant related to the initial production of enterotoxin.

The solution of Equation 1, by assuming that $y > y_c$, is given by:

$$ SEA(y) = \frac{k}{2} (y - y_c)^2 $$

The term $e^{\beta t}$ observed in Equations 1 and 2 was included to describe the fact that the bacteria start to produce a larger amount of toxins during the stationary phase of growth. As a consequence of this behavior, it is necessary to include in the model a factor independent of the microbial population that grows with time. Therefore, there were used two mathematical models in the present study: 1) The Baranyi growth model used to estimate the microbial population as a function of time, and 2) The model given by Equation 2 used to estimate the concentration of SEA produced by the bacteria. If the value of $y$ obtained after fit of the Baranyi model to experimental data is inserted in Equation 1, it is possible to directly obtain an equation expressing the concentration of SEA as a function of time. Both models were fitted to experimental data and all statistical analyses were performed with the software R v. 3.1.2.
2.2 Experimental Data

Four different published data sets were used in this work: 1) Enterotoxin A (SEA) produced by a cocktail of three *S. aureus* strains (SA79, SA161, and SA168) grown in brain heart infusion broth with 1.6 mM of undissociated lactic acid at incubation temperatures of 25ºC, 30ºC, and 37ºC, respectively (Rosengren et al., 2013); 2) SEA produced by a cocktail of three *S. aureus* strains (SA79, SA161, and SA168) grown in brain heart infusion broth with 0.2 mM of undissociated lactic acid at incubation temperatures of 25ºC, 30ºC, and 37ºC, respectively (Rosengren et al., 2013); 3) SEA produced by *S. aureus* strain 12057 grown in sterilized liquid milk at 23 ºC (Fujikawa and Morozumi, 2006); 4) SEA produced by *S. aureus* strain 12057 grown in sterilized liquid milk at 32 ºC (Fujikawa and Morozumi, 2006).

To analyze data, all data points were converted into a common measure by using the graph-digitizing software GetData Graph Digitizer 2.24. To access the published data, the graphs of interest were copied, transformed into a jpeg-format, and processed with the GetData software.

3. RESULTS AND DISCUSSION

Figure 1 presents results obtained for the first data set studied. As can be easily seen, the mathematical model predicted the concentration of SEA accurately. Therefore, it can be safely used to estimate the minimal bacterial load required to produce SEA. Roughly speaking, it can be said that the production of SEA can be divided in two stages, one linear in the first days and one close to exponential in the final days. The first stage is included in the parameter $k$ of Equation 2. On the other hand, the exponential stage of production of enterotoxins is directly affected by the parameter $\beta$ in the same Equation.

The authors of the original study employed a different approach to model the SEA production. It was used a linear model to study the SEA concentration in a smaller range (with times varying from 0 to 4 days) (Rosengren et al., 2013). Although, this approach provided very accurate results, it does not describe the toxin concentration in the range that causes illness. Thus, an alternative approach like the one used in the current study is desirable from the standpoint of food safety. In addition to these results, it can be inferred from data presented in Table 1, that the rate constants obtained for both stages of growth are higher for data set 2 in relation to data set 1. Thus, the higher the stress applied to the bacteria (1.6 mM of undissociated lactic acid in relation to 0.2 mM undissociated lactic acid), more the production of toxin will be inhibited (Wallin-Carlsquist et al., 2010).

In general, it was observed a good fit of the model to the experimental data used in the study. It can be also observed in data presented in Table 1 that the production of Enterotoxin A dramatically increases with the temperature (see the differences in the rate constants). However, a similar behavior is not observed for the parameter $y_c$, which seems not depend on the temperature. For all data sets and conditions studied the minimal *S. aureus* concentration required to produce toxins ranged from 5.8 to 7.4 independent of the temperature of exposition.
Figure 1 - Curves obtained after fit of Equation 2 to the first data set studied at (a) 25°C, (b) 30°C, and (c) 37°C.

Table 1 - Parameters obtained after fit of Equation 1 to experimental data sets.

<table>
<thead>
<tr>
<th>Data Set</th>
<th>$y_c$ (log CFU/g)</th>
<th>$k$ (ng/ml) / (log CFU/g)$^2$</th>
<th>$\beta$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (25°C)</td>
<td>6.241</td>
<td>4.03 * 10^{-1}</td>
<td>1.251 day^{-1}</td>
<td>0.990</td>
</tr>
<tr>
<td>1 (30°C)</td>
<td>7.022</td>
<td>2.94 * 10^{1}</td>
<td>1.478 day^{-1}</td>
<td>0.999</td>
</tr>
<tr>
<td>1 (37°C)</td>
<td>7.318</td>
<td>1.97 * 10^{2}</td>
<td>1.580 day^{-1}</td>
<td>0.987</td>
</tr>
<tr>
<td>2 (25°C)</td>
<td>5.964</td>
<td>6.52 * 10^{-1}</td>
<td>1.332 day^{-1}</td>
<td>0.993</td>
</tr>
<tr>
<td>2 (30°C)</td>
<td>6.125</td>
<td>7.23 * 10^{1}</td>
<td>1.496 day^{-1}</td>
<td>0.981</td>
</tr>
<tr>
<td>2 (37°C)</td>
<td>7.292</td>
<td>3.16 * 10^{2}</td>
<td>1.518 day^{-1}</td>
<td>0.985</td>
</tr>
<tr>
<td>3</td>
<td>5.836</td>
<td>7.30 * 10^{-2}</td>
<td>0.231 h^{-1}</td>
<td>0.994</td>
</tr>
<tr>
<td>4</td>
<td>6.104</td>
<td>1.41 * 10^{-1}</td>
<td>0.302 h^{-1}</td>
<td>0.992</td>
</tr>
</tbody>
</table>

An analogous behavior to the one presented in Figure 1 was observed for the other data sets studied (3 and 4), as can be seen in Figure 2. These data probably apply only to the linear stage of production of Enterotoxin A, since there were not data available for the exponential stage in these data.
sets. It can be observed in Table 1 that the rate constants increases with the temperature for data sets 3 and 4, as previously observed for data sets 1 and 2.

Figure 2. Curves obtained with a) Baranyi model applied to describe *S. aureus* growth at 32ºC b) Equation 2 applied to model SEA production. Both curves were applied to the fourth data set studied at 32ºC.

4. CONCLUSIONS

In the present study, it was proposed a mathematical model to estimate the production of Enterotoxin A by *Staphylococcus aureus* subjected to different storage conditions. The model was applied to different published data sets. In general, it was observed a good agreement between the results predicted by the model and the experimental data. Such a model can accurately describe two stages of the production of Enterotoxin A and can be regarded as a useful tool to risk assessment studies related to the production of toxins in different food products.

5. REFERENCES


