Development of Predictive Growth Models for *Staphylococcus aureus* and *Bacillus cereus* on Various Food Matrices Consisting of Ready-to-Eat (RTE) Foods

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Abstract

We developed predictive growth models for *Staphylococcus aureus* and *Bacillus cereus* on various food matrices consisting primarily of ready-to-eat (RTE) foods. A cocktail of three *S. aureus* strains, producing enterotoxins A, C, and D, or a *B. cereus* strain, were inoculated on sliced bread, cooked rice, boiled Chinese noodles, boiled bean sprouts, tofu, baked fish, smoked chicken, and baked hamburger patties at an initial concentration of 3 log CFU/g and stored at 8, 10, 13, 17, 24, and 30°C. Growth kinetic parameters were determined by the Gompertz equation. The square-root and Davey models were used to determine specific growth rate and lag time values, respectively, as a function of temperature. Model performance was evaluated based on bias and accuracy factors. *S. aureus* and *B. cereus* growth were most delayed on sliced bread. Overall, *S. aureus* growth was significantly (*p*<0.05) more rapid on animal protein foods than carbohydrate-based foods and vegetable protein foods. The fastest growth of *S. aureus* was observed on smoked chicken. *B. cereus* growth was not observed at 8 and 10°C. *B. cereus* growth was significantly (*p*<0.05) more rapid on vegetable protein foods than on carbohydrate-based foods. The secondary models developed in this study showed suitable performance for predicting the growth of *S. aureus* and *B. cereus* on various food matrices consisting of RTE foods.

Key words: *S. aureus*, *B. cereus*, animal protein foods, food matrices, predictive modeling

Introduction

Recently, the ready-to-eat (RTE) food industry has expanded due to changes in customer consumption patterns. In particular, convenience stores have been established as new distribution channels, and supply various RTE foods such as hamburgers, sandwiches, *kimbab*, and salads, which are consumed without heat processing (Park et al., 2005). In Korea, many outbreaks of foodborne disease have been attributed to the consumption of RTE foods. Foodborne illness outbreaks due to RTE foods greatly increased from 77 cases in 2002 to 354 cases in 2008. In 2009, 809 of all food poisoning cases (1,831 cases) in Korea were caused by bacteria, where 23.4% of bacterial foodborne illnesses were caused by *Staphylococcus aureus* and 3.2% by *Bacillus cereus* (KFDA, 2009). Moreover, such foodborne illnesses were mainly caused by RTE foods such as *kimbab*, a lunch box, and seasoned greens, which were prepared at retail stores (Kim et al., 2005). Recently, the Korea Food and Drug Administration has regulated the microbial standards of pathogens on RTE foods; *S. aureus* and *B. cereus* should not exceed 100 CFU/g and 1000 CFU/g, respectively (KFDA, 2008).

*S. aureus* has been recognized as an indicator of the poor hygiene of foods and processing plants, and is a major cause of food gastroenteritis worldwide (Simon and Sanjeev, 2007). *S. aureus* is a Gram-positive bacterium, commonly occurring in grape-like clusters. If environmental conditions such as time and temperature during food storage and preparation allow the growth of *S. aureus*, staphylococcal enterotoxins may be produced, offering potential harm to consumers (Sneed et al., 2004). There are several studies reporting the presence of *S. aureus* in RTE and perishable foods including raw pork or smoked ham (Atanassova et al., 2001), poultry products (Pepe et al., 2006), milk (Fujikawa and Morozumi, 2006), fish (Hiraki et al., 1998), or foods that are pre-
pared in advance before consumption and stored after preparation without adequate refrigeration (Roberts, 1986). Furthermore, the presence of S. aureus on the work surfaces and utensils of foodservice outlets has been widely demonstrated by several studies (Gibson et al., 1988; Hiraki and Suzuki, 1999). These characteristics enable S. aureus to grow and survive in a wide range of environmental conditions as well as to persist in stressful environments (e.g. dry surfaces) for long periods.

The presence of B. cereus has been reported in a variety of foods including milk and dairy products, meat and meat products, spices, dried products, cereals, especially rice, and eggs (Kramer and Gilbert, 1989). B. cereus is a Gram-positive, spore-forming, and facultative anaerobe that grows best under aerobic conditions (Granum, 1994). It is a common food contaminant and is an etiological agent of two distinct forms of illness, i.e., emetic and diarrheal. Carlin et al. (2006) observed different percentages in spore germination at chilled temperatures among B. cereus genetic groups.

A predictive model is a mathematical expression that describes the growth, survival, and inactivation of foodborne microorganisms. Such models have been used extensively to predict the safety of foods under various environmental conditions. Since the growth and survival of microorganisms are very much affected by the model media used in the research for model development, it is very important to consider a model food’s characteristics such as its food matrix and pH, which may influence the growth of microorganisms (Gibson et al., 1988). This study aimed to develop predictive growth models for S. aureus and B. cereus on various food matrices. The developed models will be used to quantify the effect of temperature on the growth of S. aureus and B. cereus on RTE foods according to the characteristics of various food matrices, as well as to determine the shelf-life of RTE foods at the retail market.

**Materials and Methods**

**Bacterial strains and preparation**

S. aureus producing enterotoxin A (ATCC 13565) and D (ATCC 23235) strains were obtained from Gyeong Sang University, and wild type, enterotoxin C was obtained from the Korea Food and Drug Administration (KFDA). B. cereus (ATCC 11778) was purchased from the Korean Federation of Culture Collections (KFCC). The S. aureus and B. cereus cultures were maintained in 10 mL of tryptic soy broth (TSB, Difco, USA) containing 20% glycerol at -80°C, respectively. The stock cultures were thawed at room temperature and then 10 μL was inoculated into a flask containing 10 mL of sterile TSB or NB under aseptic conditions. The flasks of TSB and NB were sealed with foam plugs and incubated on a rotary shaker (140 rpm) for 24 h at 35°C and 30°C under aerobic conditions, respectively. Both cultures were grown until the late exponential phase of growth (>8 log CFU/mL). Under aseptic conditions, 1 mL of each culture was serially diluted into 9 mL of 0.1% sterile peptone water (Peptone, Difco, USA) or phosphate buffer solution (PBS), respectively.

**Preparation of samples with various food matrices**

By consideration of the primary compositions of commercial RTE foods, sliced bread, cooked rice, boiled Chinese noodles, boiled soybean sprouts, smoked chicken, tofu, baked fish, and hamburger patties were prepared as model foods in the present study. The sliced bread was purchased from a local bakery and used within an hour. Sterile, precooked rice was purchased from a local grocery store and reheated in a microwave for 150 sec according to the cooking directions of the manufacture. The Chinese noodles and raw soybean sprouts were purchased from a local grocery store and were boiled in water for 6 min and 15 min, respectively. The smoked chicken was purchased from a convenience store and was cooked for 120 sec in a microwave. The tofu was purchased from a local grocery store and used within an hour. The fish (frozen pollack) and hamburger patties were purchased from a local grocery store and were baked without oil for 3 min and 4 min, respectively. Since S. aureus and B. cereus were not detected in these samples, we cut them into 10 g portions and placed them into Petri dishes under aseptic conditions.

**Measurements of pH, Aw, and salt concentration**

The pH measurements were carried out with a pH meter (IQ Scientific Instruments, USA) using 10 g of sample mixed with 90 mL of 0.1% peptone water. Water activity was determined with an AquapLab Lite meter (Decagon, USA). The water activity meter was calibrated using a calibration solution of 6 M NaCl. The salt concentration was measured by the Mohr method (AOAC, 1995). For the direct titration method, 10 g of weighed sample was placed into a 250 mL Erlenmeyer flask with 90 mL of distilled water and allowed to stand for 5 to 10 min with occasional swirling. Two milliliters of 5%