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Growth modeling of Uropathogenic *Escherichia coli* in ground chicken meat☆

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ABSTRACT

Extraintestinal Pathogenic *Escherichia coli* (ExPEC), including Uropathogenic *E. coli* (UPEC), are common contaminants in poultry meat, and are a major pathogen associated with inflammatory bowel disease, ulcerative colitis, sepsis, and urinary tract infections. The purpose of this study was to determine the growth potential of UPEC in ground chicken meat. A multi-isolate cocktail of UPEC was inoculated into ground chicken meat 10^{3-4} log CFU/g and incubated at 4, 10, 15, 22, and 30 °C. The USDA Integrated Pathogen Modeling Program (IPMP) was used to conduct mathematical modeling and validation of UPEC growth using the Huang Primary Model and the Huang Square Root Secondary Model. No growth occurred at 4 °C, while the lag phases were ca. 23.6, 11.5, 5.2, and 0.36 h at 10, 15, 22, and 30 °C. According to the model, the T_{min}, the minimum temperature for UPEC growth in ground chicken, was 5.1 °C. The growth rates (μ_{max}, ln CFU/g h^{-1}) were ca. 0.06, 0.27, 0.48, and 0.90. Approximately 83.9% of the residual errors are between ±0.5 log CFU/g, suggesting that the predictive models and the associated kinetic parameters are sufficiently accurate in predicting the growth of UPEC in ground chicken. These models have been validated and can be used in risk assessment of ExPEC in poultry meat.

1. Introduction

Extraintestinal pathogenic *E. coli* (ExPEC) are common contaminants in poultry meat, red meat, unpasteurized cheeses, fish and seafood, as well as fresh produce. (Johnson, Kuskowski, Smith, O'Bryan, & Tatini, 2005; Mitchell, Johnson, Johnston, Curtiss, & Mellata, 2015; Muller, Stephan, & Nuesch-Inderbinen, 2016; Vincent et al., 2010). It has only recently been determined the ExPEC, including Uropathogenic *E. coli* (UPEC) are associated with inflammatory bowel disease, ulcerative colitis and Crohn's Disease (Mirsepasi-Lauridsen et al., 2016). Once in the gastrointestinal (GI) tract the ExPEC are able to colonize ulcerative lesions, escape, and eventually cause sepsis. After colonization of the distal colon by ExPEC contaminated feces can accidentally transferred from the anus to the vagina and urethra where they cause urinary tract infections, cystitis, and pyelonephritis (Flores-Mireles, Walker, Caparon, & Hultgren, 2015; Nordstom, Liu, & Price, 2013; Plavsic, Stimac, & Hauser, 2013). ExPEC isolated directly from food which contain the appropriate virulence factors have been shown to cause disease in animal models (Stromberg et al., 2017; Vincent et al., 2010). Thus, there is a continuum of ExPEC/UPEC-associated disease from the upper GI tract to the urinary tract, which could be of food origin.

Between sepsis mediated deaths, ulcerative colitis, and UTI over 11 million people are affected by the ExPEC annually including ca. 750,000 cases of UC and sepsis, plus 10 million cases of UTI, at a cost of ca. $20 billion per year (Epstein, Magill, & Fiore, 2016; Jensen et al., 2015; Torio and Andrews, 2016; Vejborg, Hancock, Petersen, Krogfelt, & Klemm, 2011). Six percent of all deaths in the US (1999–2014) were attributed to sepsis originating from either the GI tract or urinary tract (Epstein et al., 2016). In contrast, Shiga toxin-producing *Escherichia coli* (STEC) are responsible for approximately 176,000 illnesses, 3700 hospitalizations, and 30 deaths in the US annually (Scallan et al., 2011). Unlike the STEC,

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there are currently no testing requirements to determine the presence of ExPEC in red meat and poultry, and ExPEC/UPEC is not considered to be an adulterant as are the STEC (USDA FSIS, 2014).

In recent years public health policy and consumer groups have requested additional information regarding the characterization, detection, and control of ExPEC in foods given the their high public health impact and the increasing frequency of antibiotic resistant ExPEC/UPEC-associated infections (Bennington-Castro, 2016; PCAST, 2014; PEW 2016; Ranjan et al., 2017). One of the questions we have been attempting to answer is: "whether food processing and preservation technologies used to control the STEC able to control the UPEC"? While there have been a large number of reports which model the growth potential of STEC in red meat, there is little if any regarding growth kinetics of UPEC. Growth modeling and predictive microbiology is a critical component of food safety risk assessments (USDA FSIS, 2012). In this research we developed and validated a growth model for UPEC in ground chicken breast meat using the Huang Primary and Secondary Models (Huang, 2014, 2013, 2008).

2. Materials and methods

2.1. Ground chicken

Ground chicken (95% lean), freshly prepared from skinless breasts was purchased from a local wholesaler (Lansdale, PA). Multiple lots of ground chicken were tested and lots with low *E. coli* levels were (<1 log CFU/g) was selected. The chicken were divided into 5 ± 0.1 g portions and packaged into filter bags (Whirl-Pak R, 7 oz, 95 mm × 180 mm × 0.08 mm, NASCO—Fort Atkinson, Fort Atkinson, Wis., U.S.A.). With the openings sealed, the filter bags containing chicken samples were frozen at −70 °C and used within 30 days. Freezing inactivates ca. an additional one log CFU/g of *E. coli*. No *E. coli* was detected in the chicken following frozen storage. No *E. coli* was detected in the chicken meat following thawing of the incubation for 18 h at 37 °C storage.

2.2. Uropathogenic E. coli (UPEC)

The *E. coli* isolates were obtained from the American Type Culture Collection (Manassas, VA). These include 700414, 700415, 700416, 700336 (Accession number ALIN 02000000), 700928 (Accession Number AR014026), and BAA-1161 (Accession number CU928163) (http://www.atcc.org), which was isolated from women with UTI and sepsis. The phylogroup and presence of ExPEC virulence factors of TA 700414 700416 were verified by polymerase chain reaction (Clermont, Christenson, Denamur, & Gordon, 2013; Johnson & Stell, 2000). ATCC 700414 - 700416 were subjected to genomic DNA sequencing and submissions to NCBI/GenBank are being prepared. Multi-isolate cocktails of the pathogens were used as recommended for appropriate validation of nonthermal processing technologies (NACMCF, 2006). The isolates were maintained on tryptic soy agar plates (4 °C) prior to experimentation.

2.3. Inoculation preparation

Each bacterial strain isolate was cultured individually in 5-ml of Tryptic Soy Broth (BD-Difco Laboratories, Sparks, MD) in a sterile 50-mL polypropylene tube at 37 °C for 18–24 h (New Brunswick, Model G34, Edison, NJ). The cocktail was made by combining 5 ml of each strain and centrifuged at 3600 × g for 10 min (1200×g, Hermle Model Z206A, Hermle Labortechnik, Germany). The pellet was re-suspended in sterile peptone water (BD-Difco Laboratories, Sparks, MD) to the original cocktail volume.

2.4. Inoculation of ground chicken

One night before the experiment, the frozen 5 g samples were retrieved from the freezer and thawed overnight in a refrigerator (4–5 °C). The thawed samples were inoculated with 0.1 mL of the bacterial cocktail, which was diluted before inoculation. The final concentration of UPEC in the ground chicken was ca. 10^{3-4} log CFU/g. The inoculated samples were pulsated for 1 min in a mechanical stomacher (Model BagMixer R-100W, Interscience Co., France) at maximum speed. Immediately after inoculation, the samples were incubated at 4, 10, 15, 22, and 30 °C. The incubating samples were periodically retrieved to enumerate UPEC. The sampling frequencies were determined by the incubation temperature, and ranged from every 0.5 h to every 24 h. Growth experiments were replicated at least 3 times at each temperature. No UPEC was detected from the control raw ground chicken samples.

2.5. UPEC enumeration

The bag containing the 5 g inoculated chicken samples was aseptically opened and 45 ml of 0.1% sterile peptone water added to obtain a 1:10 dilution. The samples were stomached for 2 min and serial diluted with 0.1% sterile peptone water before being placed (1 ml) on duplicate *E. coli* Petrifilms™ (3M, St. Paul, MN) to determine survivor counts. Use of *E. coli* petrifilms versus of non-selective media has been previously validated in our laboratory and is used by USDA FSIS for enumeration of *E. coli* (FSIS, 2014; Khosravi, Silva, Sommers, & Sheen, 2013; Sommers, Scullen, & Sheen, 2016; USDA). The Petrifilms were incubated and scored at ca. 24 h using a calibrated Petrifilm reader.

2.6. Kinetic analysis and mathematical modeling

In this study, three growth curves, representing three replicates, were obtained under each incubation temperature (10, 15, 22, and 30 °C). Two replicates from each temperature were combined and analyzed to determine kinetic parameters, including specific growth rates (μ_{max}, ln CFU/g h^{-1}) and lag time (λ, h). The other replication was set aside for validation of the models. The growth curves at 10, 15, and 22 °C exhibited lag and stationary phases, while the growth curves at 30 °C showed lag, exponential, and stationary phases. Therefore, the growth curves obtained at 10, 15, and 22 °C were analyzed to fit to the reduced Huang model (Eq. (1)), and the growth curves obtained at 30 °C were fit to the full Huang model (Eq. (2)) during primary model analysis (Huang, 2008, 2013). The effect of temperature on bacterial growth rate was described by the sub-optimal Huang Square-Root Model (Eq. (3), Huang et al., 2012). Temperature also affected the lag time of bacterial growth. Its effect was described by an empirical relationship (Eq. (4)).

In Eq. (1) and Eq. (2), Y_0 and Y are the initial and real-time bacterial concentrations in ln CFU/g. Y_{max} is the maximum cell concentration in the sample. μ_{max} is the specific growth rate (ln CFU/g h^{-1}), and λ is the lag time at a constant temperature (h). In Eqs. (3) and (4), a, α, and β are regression coefficients.

$$Y = Y_0 + \mu_{max}\left\{t + \frac{1}{4}ln\left[\frac{1+e^{-4(t-\lambda)}}{1+e^{4\lambda}}\right]\right\} \quad (1)$$

$$Y = Y_0 + Y_{max} - \ln\left\{e^{Y_0} + \left[e^{Y_{max}} - e^{Y_0}\right]e^{-\mu_{max}B(t)}\right\}$$
$$B(t) = t + \frac{1}{4}\ln\left(\frac{1+e^{-4(t-\lambda)}}{1+e^{4\lambda}}\right) \quad (2)$$