

Catfish Special Issue: Growth of Non-O157:H7 Shiga-Toxin Producing *Escherichia Coli* on Catfish Fillets

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Abstract

Shiga-toxin producing *Escherichia coli* (STECs) are emerging pathogens which have been involved in numerous foodborne illness outbreaks. In this study the ability of a multi-isolate cocktail of STEC serovars O26:H11, O45:H2, O103:H2, O111:NM, O121:H19, and O145:RM to grow on catfish fillets at refrigeration and abuse temperatures was investigated. Catfish fillet samples (10 g) were inoculated with the STEC cocktails to ca. 3 log CFU/g and incubated under aerobic conditions for up to 120 hrs. There was no STEC growth at 4°C, however, the STECs grew at 10, 15, 20 and 30°C in a temperature dependent manner, with higher growth rate being associated with higher temperature. Lag phase ranged from 15 h at 10°C to 1.75 h at 30°C. Exponential phase growth rate ranged from 0.03 log CFU/g/h at 10°C to 0.65 log CFU/g/h at 30°C. Growth curves constructed using ComBase DMfit provided a good statistical fit to the observed data, resulting in a high correlation coefficient (R^2) of 0.98. The results of this study provide information to risk assessors regarding the growth potential of the STECs on aquaculture-raised fish using catfish as a model system.

Keywords: E coli; STEC; Catfish; Growth curves

Introduction

There is growing public concern about the microbial safety of foods today. Foodborne pathogens are significant public health hazards in the United States [1-2]. Seafood has received increasing attention as a vehicle for foodborne illness in the United States. When adjusted for the annual per capita consumption of ca. 17 lbs, it is responsible for more foodborne illnesses than other meats or produce [3-6].

Shiga toxin-producing *Escherichia coli* (STEC) are a group of foodborne pathogens that have caused outbreaks and sporadic cases of human infections worldwide [7-11]. Although many foodborne infections are due to *E. coli* O157:H7, non-O157 STEC are also important pathogens and can cause human illnesses and life-threatening hemolytic uremic syndrome. Six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) now account for the majority of reported non-O157 STEC infections [10]. The emerging clinical importance of non-O157 STEC has been evaluated [11] and results conclude that these strains may account for 20 - 50% of all STEC infections in the United States [1,2,10].

Aquaculture now accounts for ca. 50% of seafood production worldwide [12]. Contamination of seafood (either wild caught or aquaculture-raised) with pathogenic *E. coli* has been known to occur, much of which is due to contaminated water sources, in many parts of the world [13-21]. In contrast, illnesses due to STECs from consumption of farm raised catfish in the U.S. are extremely rare [21-23]. While studies have examined the growth potential of *E. coli* O157:H7 and non-O157 STECs in meat [23], very little information is available on the growth potential of STEC in aquaculture-grown seafood products. Because we live in a global trade environment, which includes import and export of seafood products, it would be prudent to assess the growth potential of the STECs in seafood products.

The objective of this study was to evaluate the growth of a multi-isolate cocktail of non-O157:H7 STECs (serovars O26, O45, O103, O111, O121, and O145), using catfish fillets (*Ictalurus punctatus*) under refrigeration and temperature-abuse conditions as a model system for aquaculture-raised finfish.

Materials and Methods

Bacterial strains

Six shiga-toxin producing *Escherichia coli* isolates representing serotypes O26: H11, O45: H2, O103: H2, O111: H11, O121: H19, and O145: RM were obtained from Dr. Rob Mandrell (USDA, WRRRC, Albany, CA) via Dr. Pina Fratamico (USDA, ERRC, Wyndmoor, PA). The STECs were propagated on Brain Heart Infusion Agar (BHA, BBL/Difco Laboratories, Sparks, MD) and stored at 4°C prior to use.

Catfish fillets

Refrigerated (never frozen) catfish fillets (30 lbs of 84 g fillets, no additives) were obtained in bulk from a Mississippi-based catfish processor and shipped overnight to the Eastern Regional Research Center. The fillets were then subdivided and frozen at -20°C in sealed polypropylene bags (Uline, Inc., Philadelphia, PA). Naturally occurring *E. coli* was < 0.1 CFU/g (undetectable) which was determined using *E. coli* PetriFilms™ as described below. Background microflora (22 and 37°C) determined using Aerobic Plate Count PetriFilms were approximately 10⁴ - 10⁵ CFU/g. The catfish was allowed to thaw overnight in a refrigerator at 4°C prior to use in growth experiments.

Bacterial growth and inoculation

The procedure for inoculation and enumeration of bacteria was performed as previously described [24]. Each bacterial strain was

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cultured independently in 10 mL of Brain Heart Infusion Broth (BHIB, BBL/Difco Laboratories, Sparks, MD) in sterile 50 mL polypropylene tubes at 37°C (150 rpm) for 18 to 24 h. The STEC isolates grew to a cell density of ca. 10⁹ CFU/mL. The cultures were then combined, mixed by vortexing, and diluted in sterile 0.1% peptone water (PW-BBL/Difco Laboratories, Sparks, MD). The use of multi-isolate cocktails is recommended per the NACMCF [25]. The diluted STEC cocktail was then inoculated (0.1 mL) onto 10 g catfish fillet pieces to obtain a STEC density of ca. 10³ CFU/g. Three replicate samples were prepared for each time temperature condition. The samples were placed in sterile polynylon sample bags (Uline, Inc., Philadelphia, PA), which were then sealed and placed at the appropriate incubation temperatures (4, 10, 15, 23, 30°C), without ice.

Enumeration of bacteria

Following storage, the samples were assayed for surviving bacteria by standard microbiological procedures. Ninety mL of sterile PW was added to sample bags with 10 g of inoculated sample, mixed by stomaching for 90 s (Stomacher Mixer, Seward Co., UK). The samples were then serially diluted in PW, using tenfold dilutions, and 1 mL of diluted sample was placed onto duplicate *E. coli* PetriFilms™ (3M, Minneapolis, MN). The Petrifilms™ were then incubated ca. 24 hrs prior to enumeration. Non-inoculated samples were prepared and stored at the various storage temperatures, and no *E. coli* were detected on the PetriFilms™.

Statistical analysis

Each experiment was conducted independently twice with 3 replicates per experiment. Combase DMfit software (<http://www.combase.cc/index.php/en/>) was used to fit the observed data at all temperatures and determine the lag time, growth rate, maximum population and of the STECs on catfish fillets (Table 1) using the Baranyi and Roberts model [26-30]. Descriptive statistics, variance and graphics were completed using Microsoft Excel Office 2003 (Microsoft Corp., Redmond, WA).

Results and Discussion

Although not an issue in the U.S. [22], foodborne illness from seafood contaminated with STECs are a significant problem in many areas of the world [13-21]. To date there are few tools available to predict the risk of STEC growth in seafood as a result of either mild or severe temperature abuse. The purpose of this study was to determine the growth potential of non-O157:H7 STECs stored aerobically under various temperatures using catfish as a model system. In the current, study background microflora grew to a density of >7 log CFU/g in non-inoculated samples within three days, regardless of storage temperature. The STECs were capable of growth in the presence of background microflora at all temperatures with the exception of 4°C (Table 1).

Catfish fillets inoculated with ca. 3 log CFU/g of STEC and incubated at temperatures from 4-30°C began to show growth at different lag phases and growth rates (Table 1 and Figures 1-5). ComBase DMfit was used to fit observed data and determine lag time, growth rate, and maximum population of the STECs on catfish fillets (Table 1). The growth curves constructed using DMFit show a good fit with the observed data ($R^2 \geq 0.98$) using the model of Baranyi and Roberts [30].

According to our observations the non-O157:H7 STECs did not grow at 4°C (Figure 1). The growth potential was depressed at 4°C, as

the cell population decreased about 0.3 - 0.5 log CFU/g. This data is in agreement with that obtained in other studies which evaluated the growth potential of non-O157:H7 STECs in ground beef [25].

At 10°C the STEC growth in catfish fillets was less than 1 log CFU/g (0.29 log CFU/g) for up to 24 h after the initial inoculation, with a lag phase of 15.0 h (Table 1). After 24 h, growth increased exponentially, growth rate of 0.03 log CFU/g, with no additional growth at 96 h and

Temperature (°C)	Initial value (log CFU/g)	Lag phase (hr)	Maximum rate (log CFU/g/hr)	Final value (log CFU/g)	R-square
4	ND	ND	ND	ND	ND
10	3.76	15.0	0.03	6.59	0.98
15	3.51	11.7	0.34	8.57	0.99
22	3.51	4.63	0.34	9.91	0.99
30	4.04	1.75	0.65	10.1	0.99

Lag phase, exponential phase growth rates, stationary phase times were determined using DMFit software available through ComBase. ND= Not determined due to lack of growth.

Table 1: Estimated maximum growth rate, lag time and maximum population of non-O157:H7 Shiga-toxin producing *Escherichia coli* inoculated on catfish fillets at different temperatures.

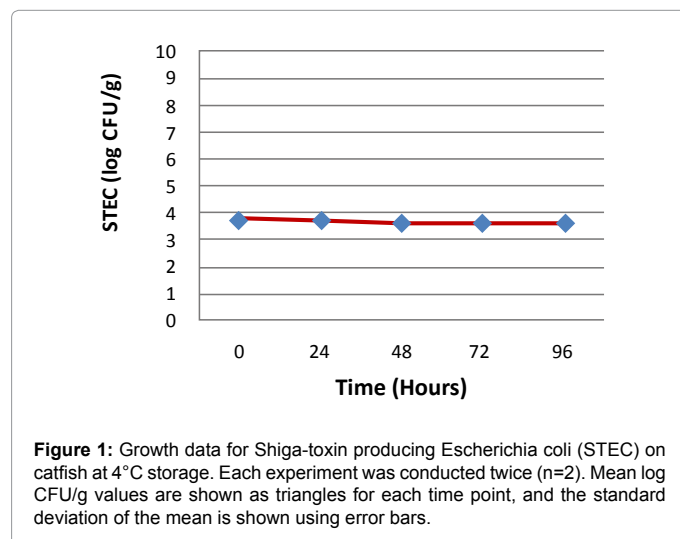


Figure 1: Growth data for Shiga-toxin producing *Escherichia coli* (STEC) on catfish at 4°C storage. Each experiment was conducted twice (n=2). Mean log CFU/g values are shown as triangles for each time point, and the standard deviation of the mean is shown using error bars.

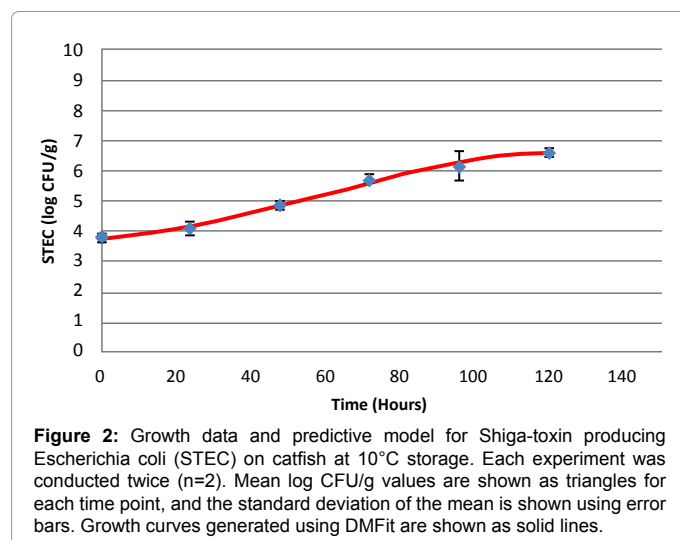
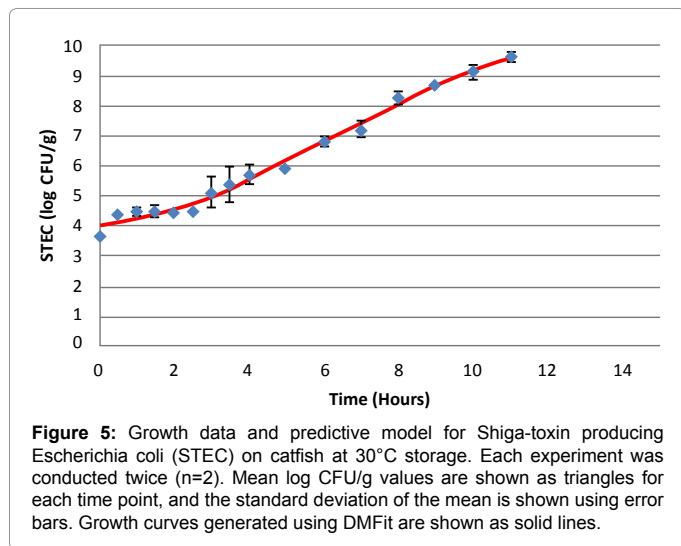
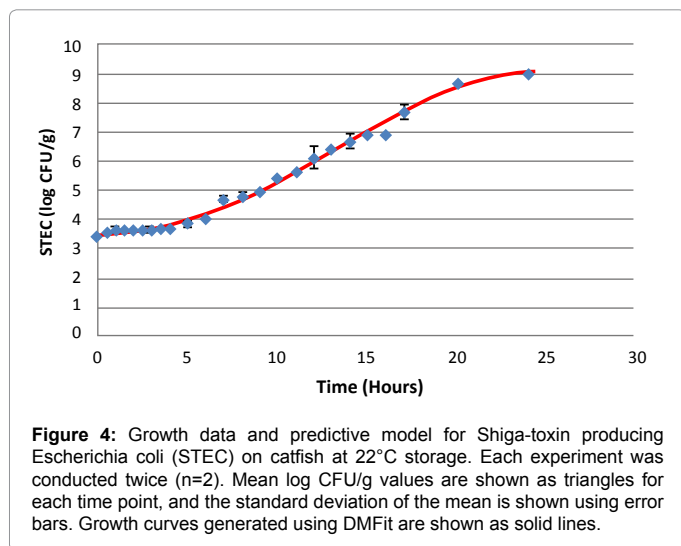
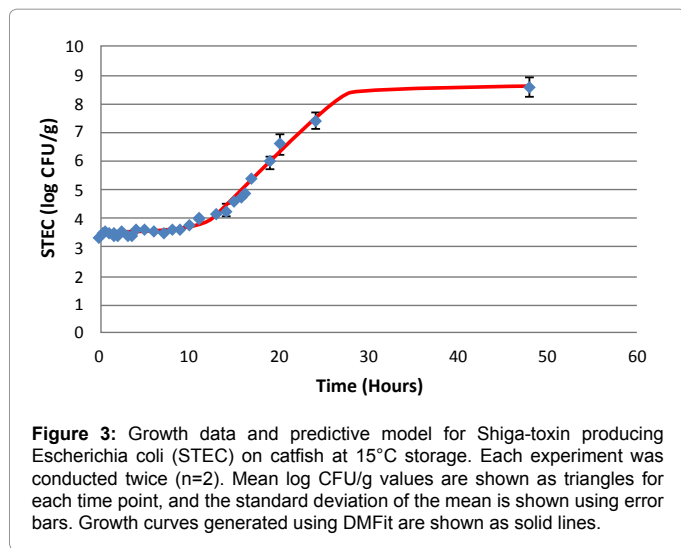


Figure 2: Growth data and predictive model for Shiga-toxin producing *Escherichia coli* (STEC) on catfish at 10°C storage. Each experiment was conducted twice (n=2). Mean log CFU/g values are shown as triangles for each time point, and the standard deviation of the mean is shown using error bars. Growth curves generated using DMFit are shown as solid lines.



120 h of incubation (stationary phase) (Figure 2). A previous study conducted using ground beef as the food matrix indicated the non-O157:H7 STECs were unable to grow in the presence of background microflora at 10°C [25]. Another study which investigated the growth potential of *E. coli* O157:H7 in ground beef reported the lag phase as 56.3 h and the exponential growth rate as 0.05 CFU/g/h. In contrast, *E. coli* O157:H7 has been demonstrated to grow at 10°C on lettuce, with an estimated lag time of 32.5 hrs prior to the start of exponential growth and an exponential growth rate of 0.02 CFU/g/h [26].

The non-O157:H7 STECs grew faster when temperature increased as determined by the observed data and fitted growth curves generated by DMfit (Figures 3-5). Except for the lowest temperature at 4°C, STEC at all temperatures, and all fitted curves exhibited lag, exponential and partially stationary phases (Figures 2-5). Lag phase decreased from 15.0 h at 10°C to 1.75 h at 30°C. Growth rate in the exponential phase increased from 0.03 at 10°C to 0.65 CFU/g/h at 30°C (Table 1).

Conclusions

Catfish fillets are a relatively short shelf-life product, even under refrigeration conditions. The non-O157:H7 STECs did not grow under proper refrigeration conditions, but were capable of growth in the presence of background microflora at a mild abuse temperature of 10°C, with a lag time significantly shorter than those for STECs on lettuce [26] and ground beef [25]. The results confirm that the growth behavior of foodborne pathogen is food matrix dependent. This information will help the seafood industry and risk assessors provide safer seafood products to consumers and guidance to the freshwater fish processing industry. These results highlight the hazards of STEC growth under conditions of mild temperature abuse in developed countries, and more severe temperature abuse which can occur in developing countries with limited refrigeration capability.

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