FITNESS IN THE BEACH SAND ENVIRONMENT: ASSESSMENT OF SAND VERSUS INTESTINAL *Escherichia coli* ISOLATES

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"In character, in manner, in style, in all things, the supreme excellence is simplicity."
Henry Wadsworth Longfellow

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ABSTRACT

FITNESS IN THE BEACH SAND ENVIRONMENT: ASSESSMENT OF SAND VERSUS INTESTINAL *Escherichia coli* ISOLATES

by William David Sadler

This dissertation examines and compares the relative fitness of Beach and Intestinal isolated *Escherichia coli* (*E. coli*) to major environmental parameters, such as: carbon, UV light, pH and temperature. A subsequent determination was made regarding, which parameters contribute to the distribution and subsequent fate of *E. coli* between primary and alternate habitats. Carbon utilization patterns were determined using a BIOLOG assay (BIOLOG, Hayward, CA). Culturability of isolates exposed to ultraviolet (UV) light, ambient drying, and extreme heat was determined by inoculation of Intestinal and Beach *E. coli* into 5 g sterile-sand flats and a 20 min exposure to 1 of 2 treatments: 1) full spectrum ambient light, or 2) ambient light >400 nm. The optical densities of cultures inoculated into wells of 96-well microtiter plates (Greiner Bio-one; Germany – via USA Scientific; Ocala, FL) were used to compare growth potentials of isolates at varying pH and temperature. Carbon utilization assays demonstrated a comparative metabolic preference of Beach isolates for carbohydrates, with the exception of D-psicose, and Intestinal isolates for amino acids. No statistical significance ($p>0.05$) was found in culturability of the isolates post UV-exposure. Beach *E. coli* achieved significantly higher growth rates ($p<0.05$) than Intestinal *E. coli* at all temperatures and pH levels tested, with the exception of pH 6.8 and 37˚C, in which differences in growth rates were not statistically significant ($p>0.05$). These findings support the theory that *E. coli*, when introduced into a beach environment, adapt and become naturalized to the environmental conditions of the alternate habitat. The importance

of these findings are realized when considering that if *E. coli* is found to be capable of adapting to and persisting in the beach environment, then its use as a fecal indicator organism is compromised.

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CHAPTER I

INTRODUCTION

Beaches are the most familiar of all coastal landforms, attracting millions of visitors each year and providing the economic base for many communities (43). In recent years, contamination of swimming water with fecal bacteria, leading to swim advisories and beach closings, has received considerable attention. However, the sand of the beach is not commonly considered in evaluating beach health, even though sand may be a suitable environment for survival of fecal bacteria such as *Escherichia coli (E. coli)* (22). In fact, beach sand may be one habitat of the freshwater environment where *E. coli* persist and perhaps grow in the Great Lakes region (2), and sand *E. coli* may be re-introduced into the swimming water (1, 42).

E. coli is an enteric bacterium that is generally described as a commensal species (15) and regarded as part of the normal lower intestinal microbiota (15, 17). Thus, the intestine of endothermic animals is considered the primary habitat for *E. coli*, the environment in which selective pressures shape the genetic structure of the population. We know a great deal about *E. coli* as a model organism for biochemistry and genetics, but we know practically nothing about the real "life" of *E. coli*. Studies on the diversity and population structure of commensal *E. coli* in the mammalian intestine are just now being explored (7, 8) and even less is known about *E. coli* that reside in extra intestinal environments such as beach sand.

The human intestine is a homeostatic environment with constant darkness and moisture, minimal pH deviation from 6.8 (25), and a narrow temperature range of 34.4˚C to 37.8˚C (36). In contrast, the Great Lakes' beach environment is exposed to a dynamic set of

environmental parameters; for example, recurring saturation and drying cycles caused by wave action, potential for higher alkalinity relative to the intestinal environment, and varying light exposure and temperatures. Yet, numerous studies have shown that beach sand can harbor significant levels of *E. coli* (12, 27) and *E. coli* has been isolated from beaches on all five of the Great Lakes. It has been suggested that Great Lakes beach sand may be a secondary habitat for *E. coli* (42, 10).

The relatively recent urbanization of the Great Lakes basin has exposed the coastal ecosystem to an increase in anthropogenic disturbances, for example pollution. Anthropogenic stressors can perturb coastal ecosystems and potentially increase the susceptibility of coastal ecosystems to non-indigenous organisms, such as *E. coli*. Sewage runoff and fecal pollution can lead to the introduction of intestinal microorganisms to the beach environment; potential sources of *E. coli* to beach sand are wildlife, livestock, and human sewage runoff. An introduction of intestinal microorganisms into the beach environment followed by persistence in the sand may allow the beach to become an alternative habitat for *E. coli*.

Certain strains of *E. coli* that posses the ability to adhere to sediment particles and take refuge in the sand may persist longer than those strains that lack an adherence mechanism (7). Sediment particles within beach sand could provide suitable microenvironments and spatial heterogeneity, both serving *E. coli* as protection from environmental stressors like ultraviolet light exposure, desiccation, and predation. Also, the organic content of detritus and silts associated with the sand has been shown, both in laboratory and *in situ*, to facilitate the persistence of these bacteria in this type of

environment for an extended period of time (2). The mechanisms by which *E. coli* survives in or adapts to the beach sand environment are poorly understood.

The apparent ubiquity of *E. coli* at Great Lakes beaches begs the question, how can native intestinal microorganisms be readily found in an environment so different from the intestine? The application of two ecological theories— 1) the source-sink model and 2) the environmental adaptation and naturalization model—provide two potential explanations for the distribution of *E. coli* between the primary and alternate habitat.

According to the source-sink model, population growth or decline is influenced by variations in the quality of the habitat (31). In applying this theory, it is assumed that the intestine would be the higher quality habitat, and the sand would be the poorer quality habitat, and there is a constant flow of *E. coli* from the highly productive intestinal (source) habitat into the less productive and less regulated beach sand habitat (sink). If sand isolates originate from an intestinal reservoir of *E. coli*, it is predicted that sand and intestinal *E. coli* populations should both contain a high genetic diversity and not be differentiated. Also, it is predicted that, in the absence of continued immigration events, *E. coli* should disappear from beach sand (sink) habitats.

The environmental adaptation and naturalization model, when applied to rapidly evolving bacterial populations, suggests the occurrence of adaptive evolution in alternative habitats. Sink-adaptive mutations could be favored in an event where large and/or sustained immigrating populations were introduced to a sink habitat, or if the quality of the sink habitat was altered, even temporarily, and the source organisms are allowed to persist. The environmental adaptation and naturalization model would predict that, if some intestinal *E. coli* strain with broad environmental tolerance was immigrating to and then adapting to

beaches, the genetic relationships among *E. coli* populations at beaches within the Great Lakes region would be more divergent from intestinal *E. coli* populations. Furthermore, if local adaptation is occurring in sand habitats, then sand-adapted *E. coli* should be more fit in the sand habitat than intestinal *E. coli*. Finally, sand *E. coli* genomes may contain additional genes acquired from other microorganisms in the local sand community, via horizontal gene transfer, resulting in the presence of geographically distinct populations.

Although much is known about the characteristics of *E. coli* when grown under artificial laboratory conditions, and recent gains in the knowledge of its lifestyle in the mammalian intestine, little work has been done from the perspective of considering *E. coli* to be an organism adapted to an extra-intestinal habitat. It is hypothesized that with respect to carbon utilization, UV light, pH and temperature growth tolerance, that there will be significant differences between the growth rates of *E. coli* isolated from intestines and *E. coli* isolated from beach sand, suggesting they are different populations. The goal of this study was to compare beach isolated *E. coli* to intestine isolated *E. coli* with regard to fitness in the beach sand habitat by comparing relative tolerances of each to variances in pH, temperature, UV light, and ambient drying, and to compare carbon utilization patterns.

CHAPTER II

MATERIALS & METHODS

Sample Isolation

Beach sand *E. coli*

Ten *E. coli* previously isolated from the top 1-5 cm of sand at Lake Huron beaches were retrieved from frozen glycerol stocks generated during previous studies of the Alm Lab (Table 1). The Lake Huron isolates used in this study were characterized by Walk *et al.* 2007 and were found to be of a persistent environmental genotype that grouped into a distinct phylogenetic clade within the diversity of *E. coli*. The *E. coli* within this clade may possess unique traits that increase their fitness to conditions in the freshwater beach sand environment (39). These beach *E. coli* isolates were revived from frozen stocks on Nutrient Agar and additional glycerol stocks were prepared for use in this study.

Table 1. Lake Huron Beach *E. coli* isolates.

Isolate ID	**Beach**	**Isolation Date**
B(1)	Conger Lighthouse	8/20/02
B(2)	Marysville	8/20/02
B(3)	Lake Port Camp	7/9/02
B(4)	Lake Port Camp	8/20/02
B(5)	Lake Port Camp	9/6/02
B(6)	Marine City	7/25/02
B(7)	Marine City	9/6/02
B(8)	Lake Port Beach	7/25/02
B(9)	Lake Port Beach	8/20/02
B(10)	Lake Port Beach	9/6/02

Intestinal *E. coli*

A sequence of selective plating followed by biochemical characterization was used to isolate *E. coli* from the feces of human volunteers. Feces were collected using sterile swabs (Thermo Fisher Scientific, Waltham, Massachusetts) and the inoculated swabs were streaked onto Eosin Methylene Blue (EMB) agar plates (Difco, Detroit, MI). After streaking, the EMB agar plates were incubated at 37±0.5˚C for 18-24 hrs. EMB is selective for fecal colifom bacteria as a group, and *E. coli* typically form distinctive metallic green colonies (23). Isolated colonies exhibiting a green-metallic coloring, presumptive of *E. coli* were picked, and transferred onto Nutrient Agar (NA) and MacConkey agar (MAC) plates (Difco, Detroit, MI), and incubated at 37±0.5˚C for 18-24 hrs.

MacConkey agar is differential for lactose fermentation, one biochemical characteristic of *E. coli*. *E. coli* are also negative for the enzyme cytochrome C oxidase and positive for the enzyme tryptophanase. One isolated colony from each NA plate was used to inoculate a tube of sterile Tryptone broth (Difco, Detroit, MI). The Tryptone broth was incubated at 37±0.5˚C for 18-24 hrs and subsequently exposed to Indole Reagent. Production of a red ring in this test indicates the production of typtophanase. A spot Oxidase test was also performed to confirm absence of oxidase.

Intestinal isolates were identified as *E. coli* by possessing all of the following characteristics; green-metallic color on EMB, positive lactose fermentation on MAC, presence of tryptophanase, and absence of cytochrome C oxidase. These intestinal *E. coli* isolates were transferred to 2 ml microcentrifuge tubes containing 1 ml TSB+15% glycerol. The glycerol tubes were incubated at 37±0.5˚C for 4 hrs and then stored at -80˚C.

Comparison of Carbon Source Use Between Intestinal and Beach *E. coli*

Carbon source utilization patterns between Beach *E. coli* and Intestinal *E. coli* were compared. Carbon utilization patterns were determined using a BIOLOG assay. The BIOLOG microplate carbon utilization assay (BIOLOG, Hayward, CA) is based on the oxidation of carbon sources and the subsequent detection of the reduced form of a tetrazolium dye. The BIOLOG software (BIOLOG, Hayward, CA) subtracts the reading of a control well from the readings of the carbon source wells and expresses the individual well's behavior as a percent change with respect to the control well, A1. The BIOLOG system (BIOLOG, Hayward, CA) determines the ability of *E. coli* to grow on a variety of single carbon sources and results in carbon utilization pattern that can be used to assess the potential functional diversity of the *E. coli* (21).

E. coli isolates were recovered from glycerol stocks by streaking them onto Tryptic Soy Agar (TSA) plates and incubating at 37±0.5˚C for 18-24 hrs. One isolated colony from each TSA plate was then transferred to a BIOLOG Universal Growth agar + blood (BUG+B) plate and incubated as described above. Prior to inoculation of the Gram-negative/Gram-Positive Inoculating Fluid (GN/GP-IF) (BIOLOG; Hayward, CA), sodium thioglycolate (BIOLOG; Hayward, CA) (5mM) was added to each GN/GP-IF tube. Sodium thioglycolate is an anti-capsule agent that inhibits false-positive results in negative control wells, caused when bacteria utilize their own polysaccharide capsules as a carbon source. Select colonies of the respective isolate were used to bring the GN/GP-IF to 61±2% transmittance via the BIOLOG Turbidimeter (BIOLOG; Hayward, CA). The GN/GP-IF (150µl) was then inoculated into each well of the prewarmed BIOLOG GN2 microplates. BIOLOG GN2 microplates were used to detect carbon utilization on 95 different substrates.

Each microplate included a negative control (well A1). Microplates were incubated under ambient atmosphere for 18-24 hrs at 37±0.5˚C and were then read at 4-6 hrs and 20-24 hrs using the BIOLOG Microlog software and plate reader.

Data was collected from the plate reader and recorded in a Microsoft Excel 2008 (Microsoft; Seattle, WA) spreadsheet. PASW 18 Statistics (IBM Corporation; Somers, NY) was used to perform a 2-Sample T-test was used to assess statistical significance.

Influence of UV light, in combination with Ambient Drying and Heat, on Subsequent Culturability of Intestinal versus Beach *E. coli*

Culturability, being the ability to culture, of isolates exposed to UV, ambient drying, and ambient heat was determined by inoculation of Intestinal and Beach *E. coli* isolates into 5 g sterile-sand flats. UV exposure experiments took place on roof of Brooks Hall at Central Michigan University, Mt. Pleasant, MI on August 19th, 2009.

Beach sand was obtained from the shore of Lake Michigan at Whitehall, MI and transported to the laboratory in a clean 5 gallon plastic bucket, lined with a new plastic bag. Sand was homogenized with a sterile wooden spoon and autoclaved on "Dry" cycle for 30 min and allowed to rest overnight at 4˚C. Homogenization and autoclaving were repeated and sand was allowed to cool to room temperature. Sterile sand (5 g) was aseptically transferred to sterile 47mm Petri dishes (Thermo Fisher Scientific, Waltham, Massachusetts).

The Intestinal and Beach *E. coli* isolates were recovered from glycerol stocks by streaking them onto NA plates and incubating for 18-24 hrs at 37±0.5˚C. One isolated colony from each plate was picked and transferred, via sterile inoculating needle, into 10 ml of room temperature Tryptic Soy Broth (TSB) (Difco, Detroit, MI). Isolates were then

incubated overnight at 37±0.5˚C at with shaking at 140 rpm. Cells were pelleted by 6000 rpm centrifugation for 7 min using a Sorvall RT6000, supernatant was decanted, and cells were resuspended in sterile 0.85% NaCl. Centrifugation, decantation, and resuspension were repeated a final time in 0.85% NaCl and then adjusted to a $1.5x10^8$ cells/ml estimated by comparison to a McFarland Equivalence Turbidity Standard (Remel, Lenexa, KS). The *E. coli* were then diluted to a final concentration of $1.5x10^3$ cells/ml and used to inoculate duplicate 5 g sterile-sand flats for each Intestinal and Beach isolate.

Inoculated sand flats were divided into two groups, "UV Exposure" and "Non-UV Exposure". Treatment of the "UV Exposure" group was one 20 min exposure to ambient daylight and ambient-rate drying, while the "Non-UV Exposure" group treatment was one 20 min exposure to ambient visible light and ambient rate drying. A 121 cm x 0.317cm x 91.4 cm sheet of UF-5 Plexiglas® (Laird Plastics, Bensenville, IL) was suspended 14 cm above a 121 cm x 91.4 cm plywood base using a combination of metal bolts, washers and nuts for support. UF-5 Plexiglas® allows the transmission of visible light (>400 nm), while absorbing ultraviolet light (<400 nm) and was used to restrict UV light transmission to the "Non-UV Exposure" sand flats. "UV Exposure" sand flats were not restricted in their exposure to daylight. Negative control plates (5 g uninoculated sterile sand flats) and dark exposure control plates (*E. coli* inoculated 5 g sand flats that were not exposed to visible or UV light) were included in the experiment. Random interspersion was employed during treatment exposure. Post-treatment sand flats were allowed to rest over night at room temperature. Enumeration of *E. coli* occurred on the following day.

At T = 0, 5 g of sand was taken for *E. coli* enumeration, which was used to provide an initial concentration that allowed for the quantitative assessment of isolate culturability

post-treatment. *E. coli* enumeration was accomplished using a modified version of the U.S. Environmental Protection Agency (USEPA) Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC). Experimental sand flats were transferred to sterile 250 ml flasks and diluted in roughly 100 ml sterile water, forming sand slurry. Sand slurry was agitated @ 270 rpm for 30 sec and supernatant was vacuum filtered onto sterile 47mm membrane filters (0.45±0.02 μm pore size) (Millipore, Bedford, MA). Membrane filters were then aseptically transferred to Modified mTEC agar (Difco, Detroit, MI) plates and incubated for 2 hrs at 25±0.5˚C then overnight at 44.5±0.5˚C. Modified mTEC agar (Difco, Detroit, MI) is a selective and differential medium used for the chromogenic detection and enumeration of thermotolerant *E. coli* and conforms with U.S. Environmental Protection Agency (USEPA) Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC). After the incubation period *E. coli* colonies were counted.

PASW 18 Statistics (IBM Corporation; Somers, NY) was used to perform a 2-sample T-test to assess the presence of statistical significance in culturability after UV and ambient heat exposure in Beach and Intestinal *E. coli*. This test is appropriate because the assumption of independent samples will not be violated.

Influence of pH and Temperature on Growth Rates of Intestinal versus Beach *E. coli*

The influence of both pH and temperature on the growth rates of Beach and Intestinal *E. coli* were evaluated. The optical densities of cultures inoculated into wells of 96-well microtiter plates (Greiner Bio-one; Germany – via USA Scientific; Ocala, FL)

containing Luria Broth (Thermo Fisher Scientific, Waltham, Massachusetts) were used to compare growth potentials of isolates at varying pH and temperature. Growth conditions exposed isolates to a range of pH and temperatures that span conditions typical of both the colon and the beach: pH 6.0, 6.8 (human colon), 7.0, 8.0, and 9.0 and constant incubation temperatures of 4˚C (refrigeration), 19˚C (average summer sand temperature, (1)), and 37˚C (average human body temperature). The determination of optical density was made at 540 nm using the BioRad 550 (Hercules, CA) automated plate reader at time (T) = 0, 2, 6, 10, and 24 hours; absorbance of wells was compared to the absorbance of the respective wells' optical density at T=0 and the difference recorded (37). Each isolate was exposed to each treatment with two replicates. Replicates were present on separate microtiter plates.

Absorbance measurements were collected from the plate reader and recorded in a Microsoft Excel 2008 (Microsoft; Seattle, WA) spreadsheet. An increase in turbidity during the incubation period of at least 0.050 absolute absorbance units (AAU; Log_{10} baseline measurement intensity/sample measurement intensity) was considered "growth". Any decrease, no change, or increase less than 0.050 AAU was considered "no growth".

A repeated measures 2-way analysis of variance (repeated measures 2-way ANOVA) was used for statistical analysis; the reasoning being that the samples were dependent and violated the ANOVA assumption of independence.

CHAPTER III

RESULTS

Comparison of Carbon Source Use Between Intestinal and Beach *E. coli*

If *E. coli* is responsive to selective pressures present in the beach environment, carbon source utilization may be a parameter where differences can be observed between Beach and Intestinal *E. coli*. At the 6 hr incubation mark, a statistically significant ($p<0.05$) difference was seen between the ability of Beach and Intestinal isolates to metabolize the following carbon sources: glycogen, L-arabinose, L-fucose, D-melibiose, D-psicose, D-raffinose, L-rhamnose, acetic acid, D-gluconic acid, ρ-hydroxyphenylacetic acid, succinic acid, bromosuccinic acid, bromosuccinic acid, L-asparagine, glycyl-L-aspartic acid, D-serine, L-serine, and inosine (Figure 1). Beach *E. coli* were shown to more rapidly metabolize glycogen, L-arabinose, L-fucose, D-melibiose, D-raffinose, L-rhamnose, D-gluconic acid, ρ-hydroxyphenylacetic acid, and inosine when compared to the Intestinal *E. coli*. Acetic acid, succinic acid, bromosuccinic acid, bromosuccinic acid, L-asparagine, glycyl-L-aspartic acid, D-serine, and L-serine were more promptly metabolized by Intestinal *E. coli* than by Beach *E. coli.*

Measurements at the 24 hr incubation point revealed significant differences in metabolism between Beach and Intestinal *E. coli* isolates for the following carbon sources: D-psicose, ρ-hydroxyphenylacetic acid, D,L-lactic acid, succinic acid, glucuronamide, L-alanine, L-asparagine, glycyl-L-aspartic acid, D-serine, L-serine, and D,L-α-glycerol phosphate (Figure 2). Beach *E. coli* appeared to be capable of more quickly metabolizing ρ-hydroxyphenylacetic acid at the 24hr time point, when compared with Intestinal *E. coli*.

However, the Intestinal *E. coli* more readily metabolized D-psicose, D, L-lactic acid, succinic acid, glucuronamide, L-alanine, L-asparagine, glycyl-L-aspartic acid, D-serine, L-serine, and D, L-α-glycerol phosphate, than did the Beach *E. coli.*

When comparing Figures 1 and 2, it is noticed that the carbon sources that the Beach *E. coli* were able to metabolize further, at the 6 hr time point, were not statistically significant *($p>0.05$)* at the 24 hr time point, with the exception of ρ-hydroxyphenylacetic acid. Moreover, four new carbon sources became significantly different in their metabolism at 24 hrs: D, L-lactic acid, glucuronamide, L-alanine, and D, L-α-glycerolphosphate; Intestinal *E. coli* being able to more quickly metabolize all four of them. The Beach isolates showed higher initial metabolism of the carbohydrate carbon sources, with exception of D-psicose, while the Intestinal isolates showed consistently higher metabolism of the amino acid carbon sources.

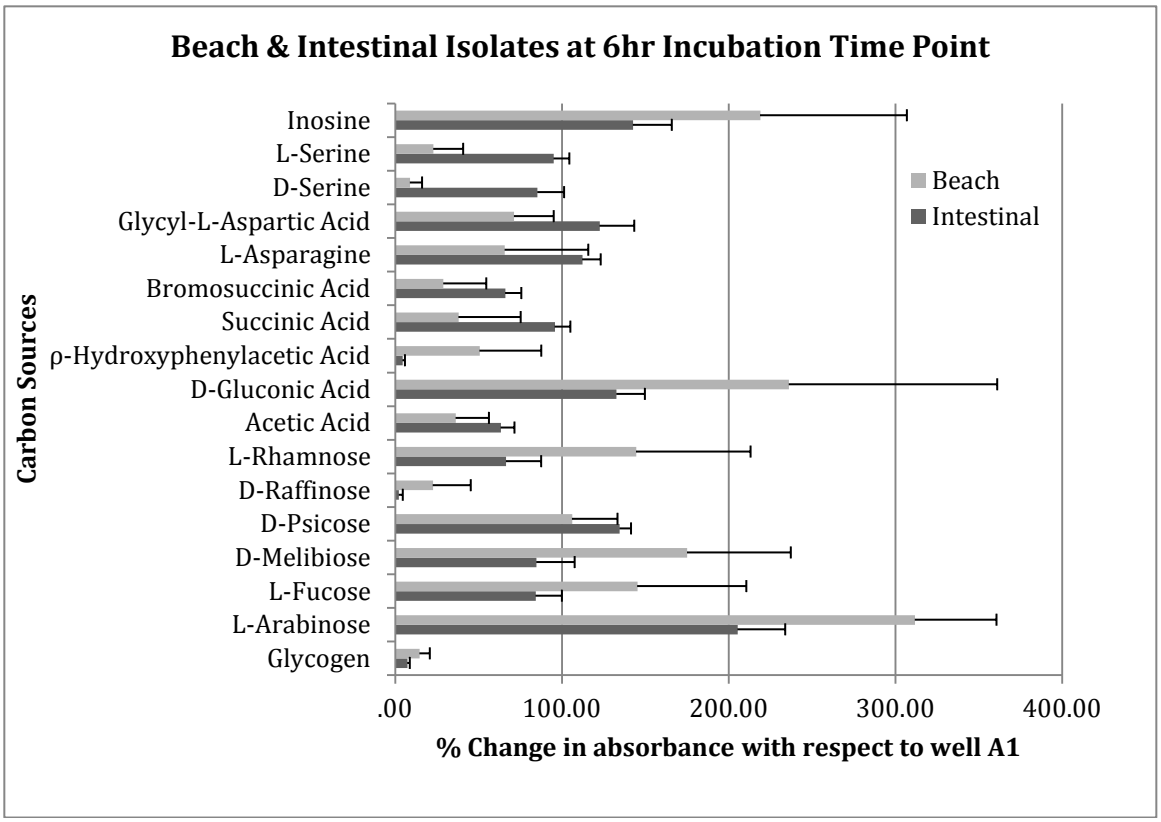

Figure 1. Mean percent change in absorbance, with respect to well A1, of carbon sources metabolized significantly differently at 6hr time point for Beach and Intestinal *E. coli* isolates. Error bars indicate standard deviation.

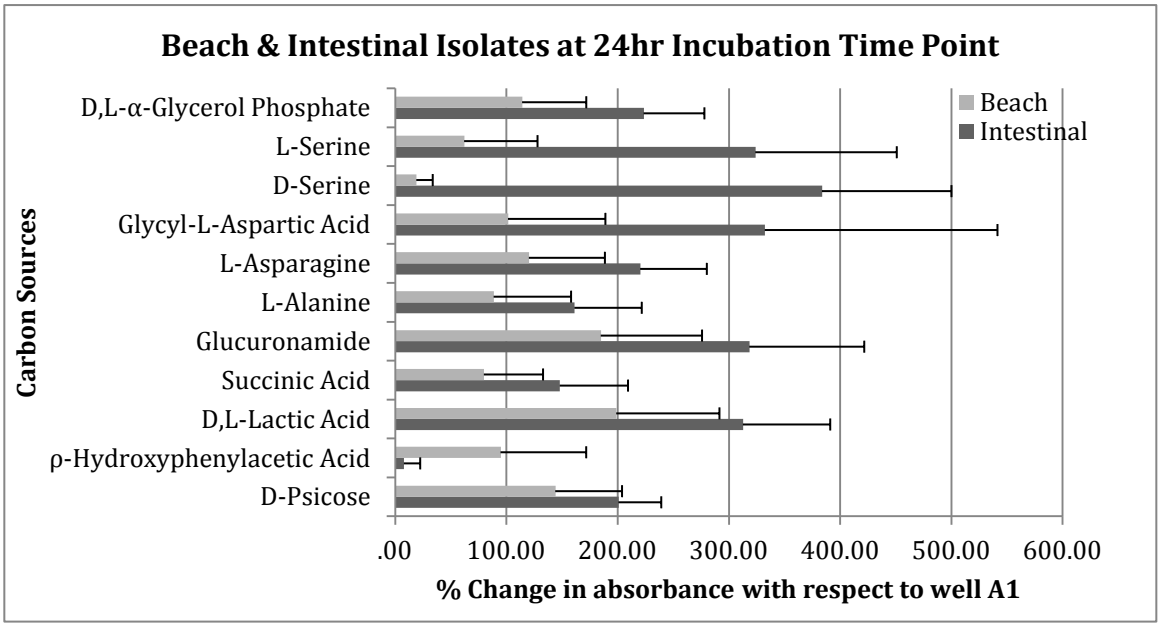

Figure 2. Mean percent change in absorbance, with respect to well A1, of carbon sources metabolized significantly differently at 24hr time point for Beach and Intestinal *E. coli* isolates. Error bars indicate standard deviation.

Influence of UV light, in combination with Ambient Drying and Heat, on Subsequent Culturability of Intestinal versus Beach *E. coli*

Observed differences in how well *E. coli*, isolated from beach sand and the human intestinal environment, cope with the combined stresses of UV exposure, ambient heat, and ambient drying, may provide insight into whether adaptation to the beach sand habitat is occurring. At the commencement of the experiment, 11:00 am on August 19th, 2009, and throughout the 20 min experimental period, weather was sunny (7.23 Kfc – 7.58 Kfc), cloud cover absent, intermittent light breeze out of the southwest, ambient air temperature of 27°C, and rooftop surface temperature of 38°C.

Following the overnight dark recovery period, two of the ten Beach *E. coli* isolates exposed to UV each produced three CFU, for a total of 6 colonies (Table 2). None of the 10 Intestinal *E. coli* exposed to UV produced colonies. The Non-UV exposed group contained no viable isolates from either the Beach or Intestinal *E. coli* after the overnight dark recovery period (Table 3).

A 2 sample T-test was performed on the viability data generated from the experiment to test for the equality of means between the Beach and Intestinal *E. coli*. As Table 3 shows, the Levene's Test for Equality of Variances proves the assumption of Equal Variances was violated. Thus, Equal Variances are not assumed in the T-statistic, degrees of freedom, 2-tailed p-value, mean difference. Furthermore, output from the 2 sample T-test shows there to be no statistical significance *($p>0.05$)* between the two groups of *E. coli* in survivability of UV and ambient heat exposure.

Table 2. Number of culturable Beach and Intestinal *E. coli* isolates after 20 min full or visible-only spectrum daylight exposure followed by overnight dark recovery.

	UV Exposed		*Non-UV Exposed*	
Isolate Type	**# Culturable Isolates**	**# Culturable Colonies**	**# Culturable Isolates**	**# Culturable Colonies**
Beach	2	6	0	0
Intestinal	0	0	0	0

Table 3. Results from T-test for Equality of Means. Equal variances not assumed.

Levene's Test for Equality of Variances						
F	**Sig.**	**t**	**df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**
10.688	0.002	1.453	19.000	.163	.30000	.20647

Influence of pH and Temperature on Growth Rates of Intestinal versus Beach *E. coli*

If *E. coli* are adapting to the beach sand habitat, those adaptations may be reflected in growth rates that are influenced by pH and/or temperature. A repeated measures two-way ANOVA, tested for significant differences between the growth rates of Beach and Intestinal *E. coli* isolates. Statistically significant differences in growth rates over a 24 hr time period were found between Beach and Intestinal *E. coli* isolates incubated at 19˚C and 37˚C and all pH levels tested, with the exception of pH 6.8 at 37˚C (Table 4 & 5).

When pH was varied and temperature was held constant at 19˚C, Beach *E. coli* consistently achieved higher and statistically significant (Table 4) growth rates than did Intestinal *E. coli*; the difference being most pronounced at the 24 hr time point (Figure 3-7).

When pH was varied and temperature was held constant at 37˚C, Beach *E. coli* still achieved higher and statistically significant (Table 5) growth rates than Intestinal *E. coli*, with the exception of pH 6.8, in which differences in growth rates between Beach and Intestinal isolates were not statistically significant (Table 5).

Regardless of pH, the isolates incubated at 19˚C (Figure 3-7) achieved higher 24 hr absorbencies when compared to the 37˚C incubated isolates (Figure 8-12). Microtiter plate readings from the 4˚C incubation revealed no detectable growth at this temperature.

In conducting the repeated measures 2-way ANOVA, the Mauchly's Test of Sphericity indicated the assumption of sphericity had been violated (Table 4 & 5). However, PASW 18 includes multiple tests, which decrease the degrees of freedom to correct for the violation. The Greenhouse-Geisser corrective test was chosen because it was neither the most conservative nor liberal correction; therefore, it was the least likely to commit a Type I or Type II error.

Table 4. Output for 2-Way Repeated Measures ANOVA of Beach and Intestinal *E. coli* incubated at 19˚C and exposed to various pH.

	Mauchly's Test of Sphericity	**Greenhouse-Geisser**	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**P-Value**
pH 6	0.000	0.285	0.058	1.14	0.051	5.882	0.016
pH 6.8	0.000	0.288	0.043	1.151	0.038	5.247	0.023
pH 7	0.000	0.318	0.055	1.270	0.043	8.138	0.004
pH 8	0.000	0.328	0.047	1.311	0.036	8.299	0.003
pH 9	0.000	0.371	0.049	1.484	0.033	17.674	0.000

Table 5. Output for 2-Way Repeated Measures ANOVA of Beach and Intestinal *E. coli* incubated at 37˚C and exposed to various pH.

	Mauchly's Test of Sphericity	Greenhouse-Geisser	Type III Sum of Squares	df	Mean Square	F	P-Value
pH 6	0.000	0.357	0.055	1.430	0.038	7.978	0.003
pH 6.8	0.000	0.385	0.026	1.54	0.017	1.226	0.292
pH 7	0.000	0.408	0.054	1.633	0.033	12.840	0.000
pH 8	0.000	0.551	0.026	2.203	0.012	12.214	0.000
pH 9	0.000	0.652	0.019	2.606	0.007	8.262	0.000

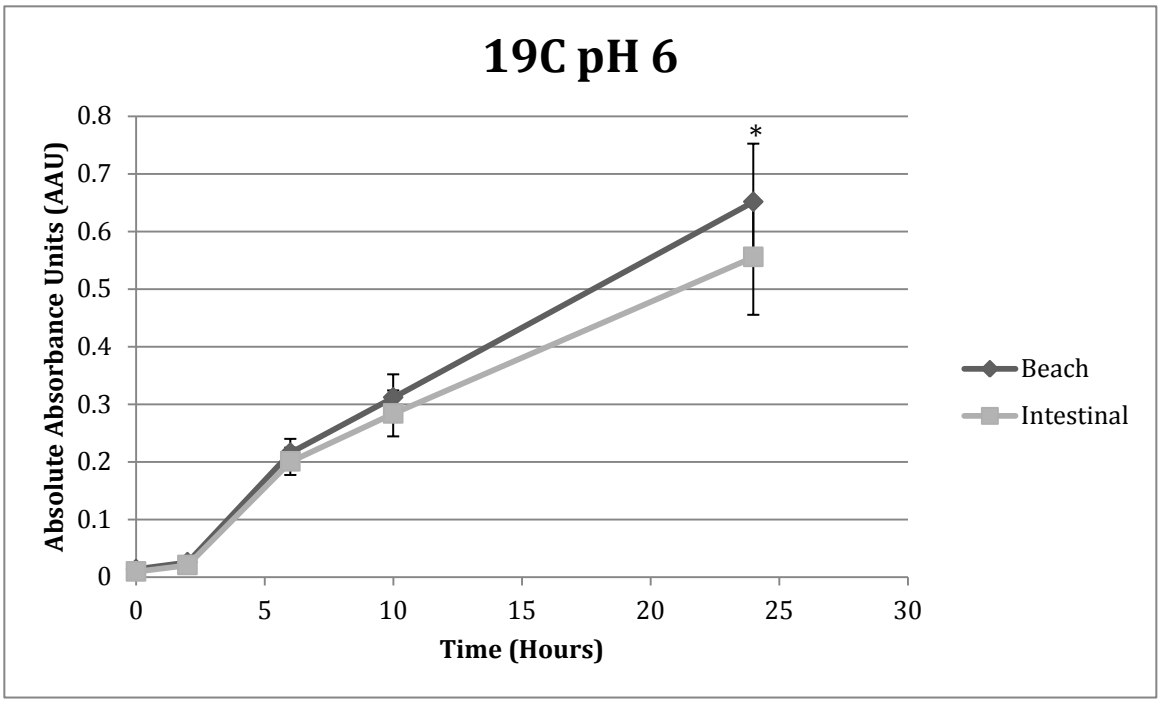

Figure 3. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 19˚C and cultured in Luria Broth adjusted to pH 6. Error bars indicate standard deviation.

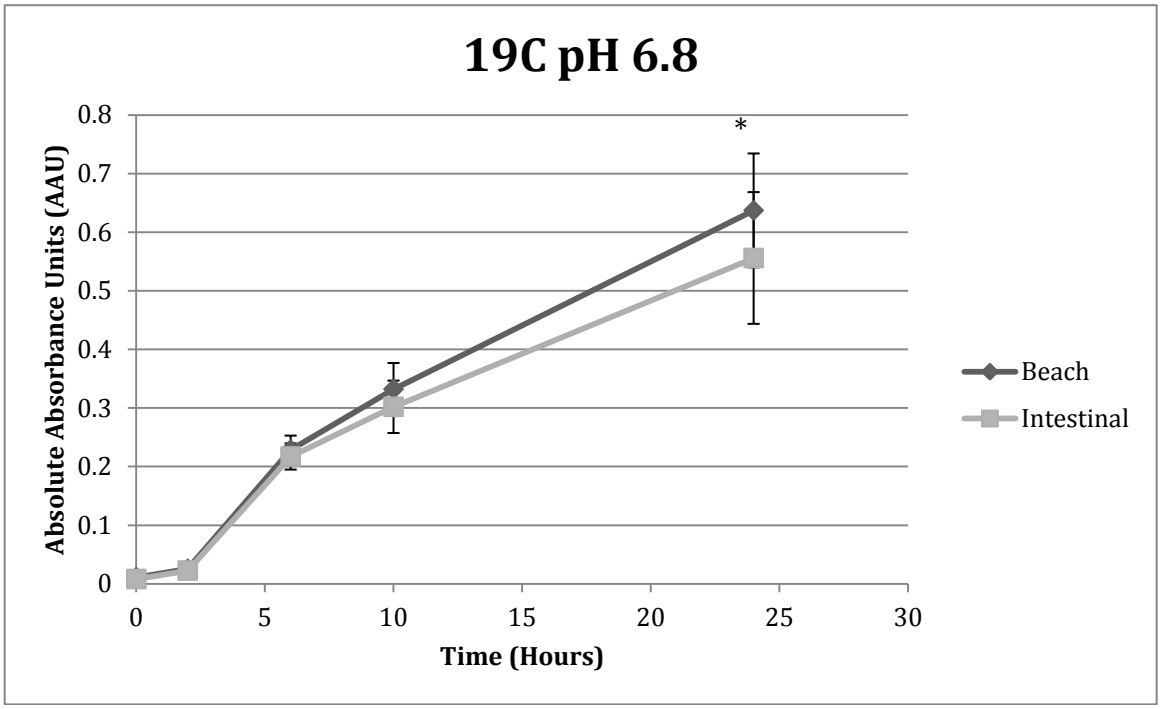

Figure 4. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 19˚C and cultured in Luria Broth adjusted to pH 6.8. Error bars indicate standard deviation.

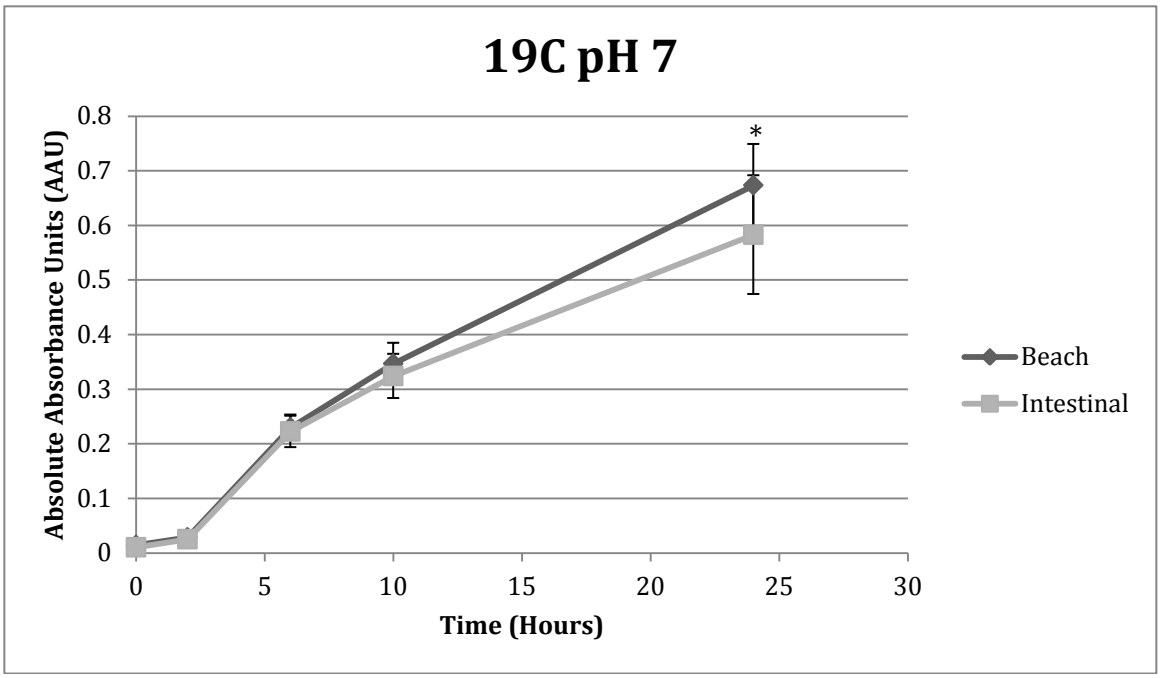

Figure 5. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 19˚C and cultured in Luria Broth adjusted to pH 7. Error bars indicate standard deviation.

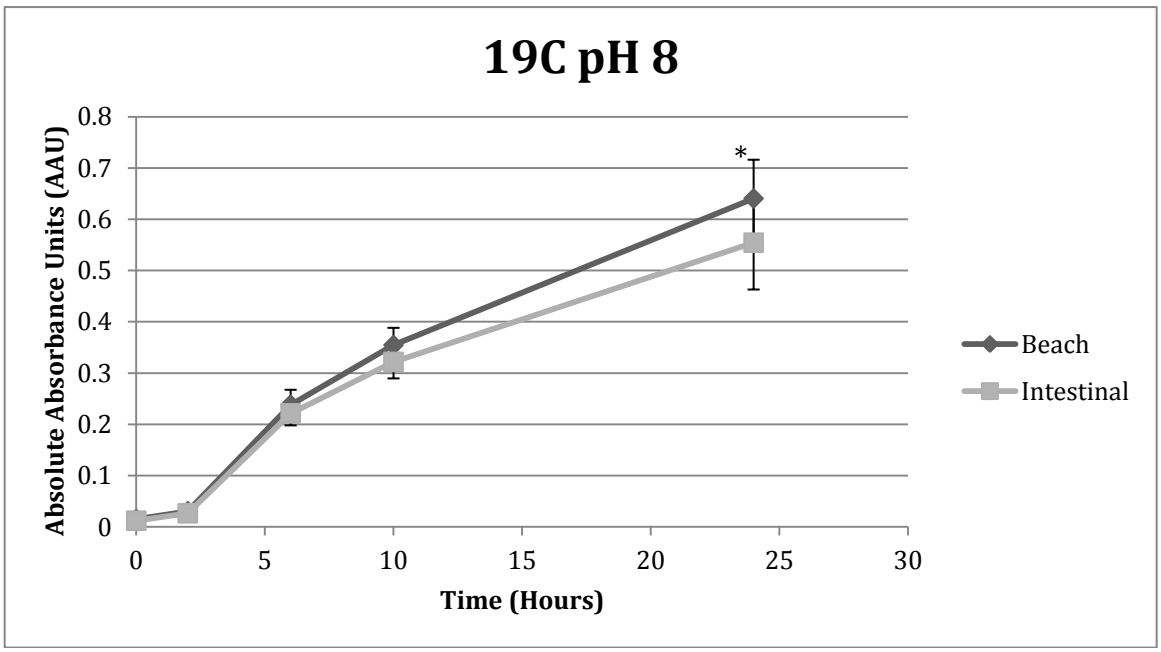

Figure 6. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 19˚C and cultured in Luria Broth adjusted to pH 8. Error bars indicate standard deviation.

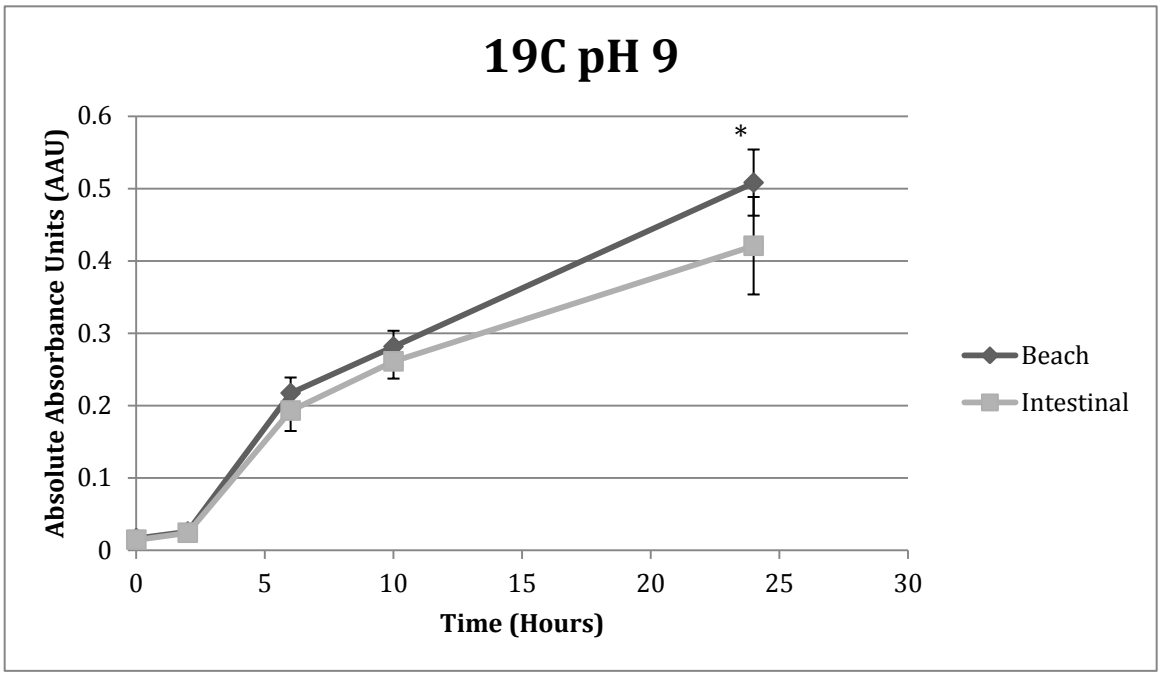

Figure 7. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 19˚C and cultured in Luria Broth adjusted to pH 9. Error bars indicate standard deviation.

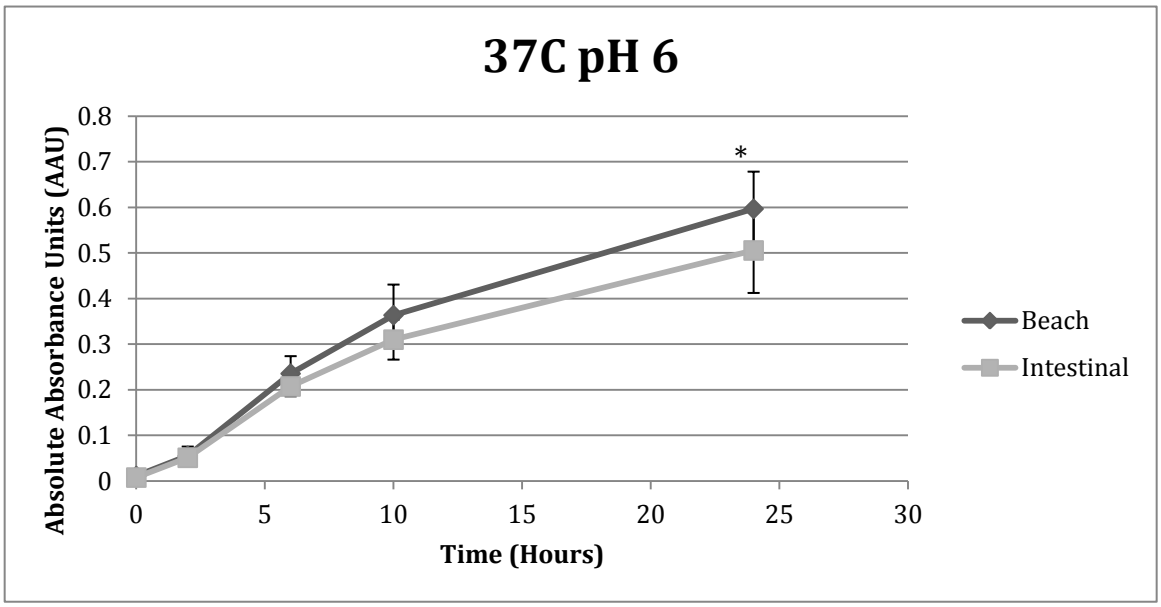

Figure 8. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 37˚C and cultured in Luria Broth adjusted to pH 6. Error bars indicate standard deviation.

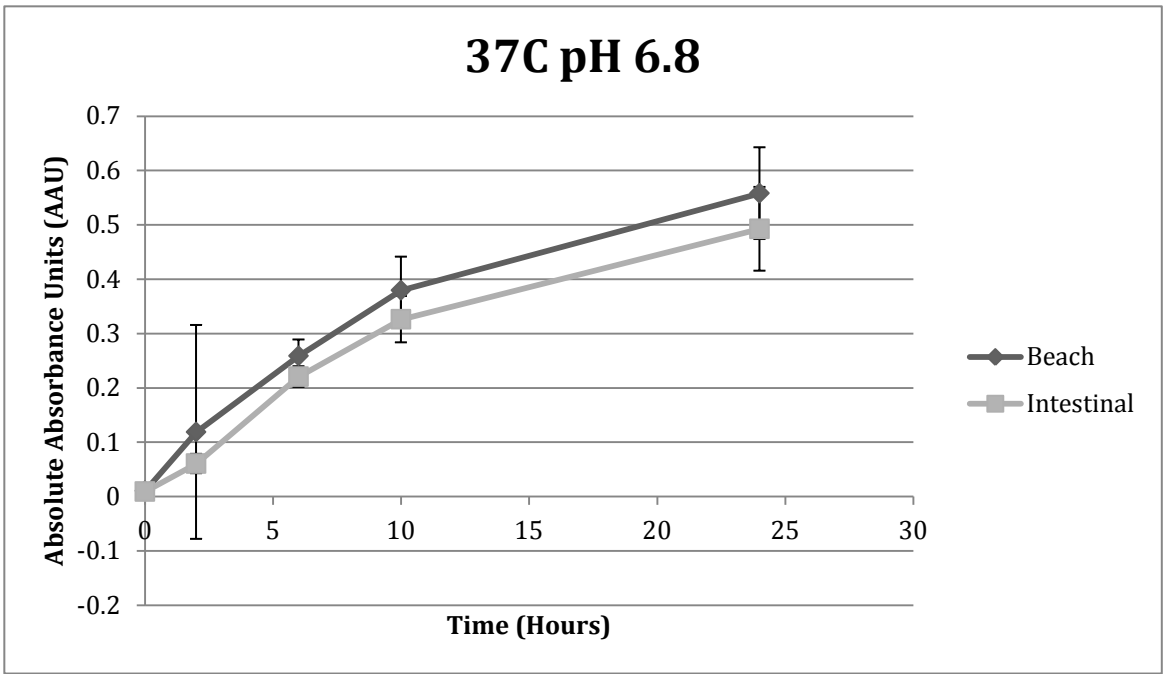

Figure 9. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 37˚C and cultured in Luria Broth adjusted to pH 6.8. Error bars indicate standard deviation.

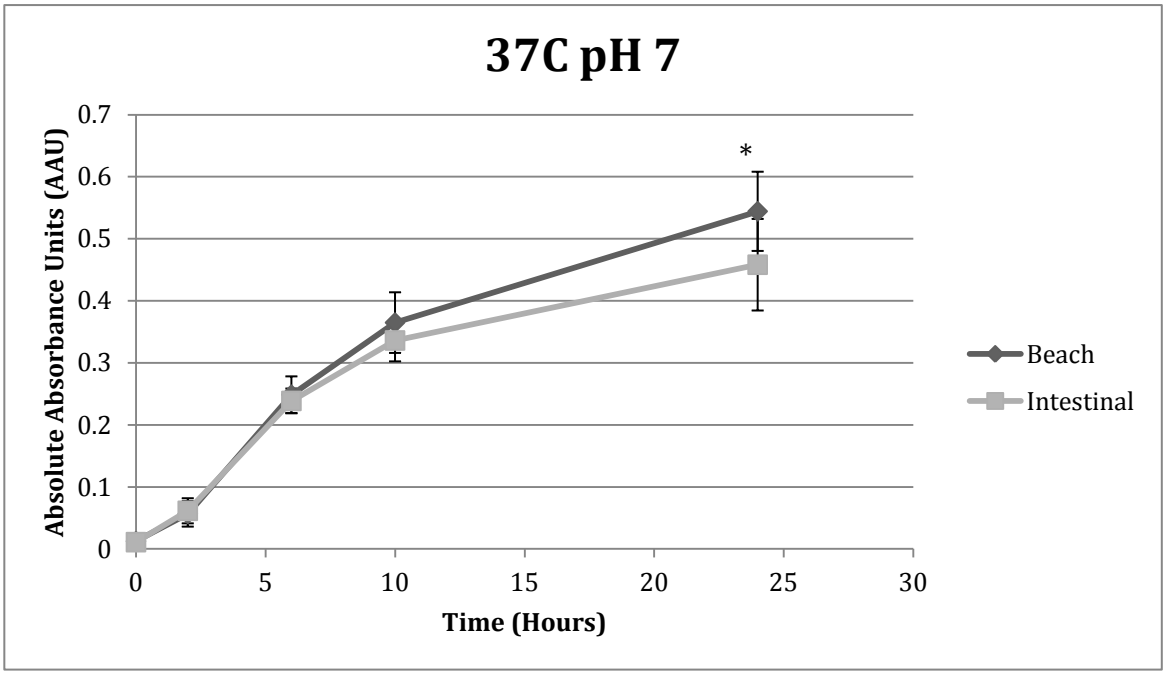

Figure 10. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 37˚C and cultured in Luria Broth adjusted to pH 7. Error bars indicate standard deviation.

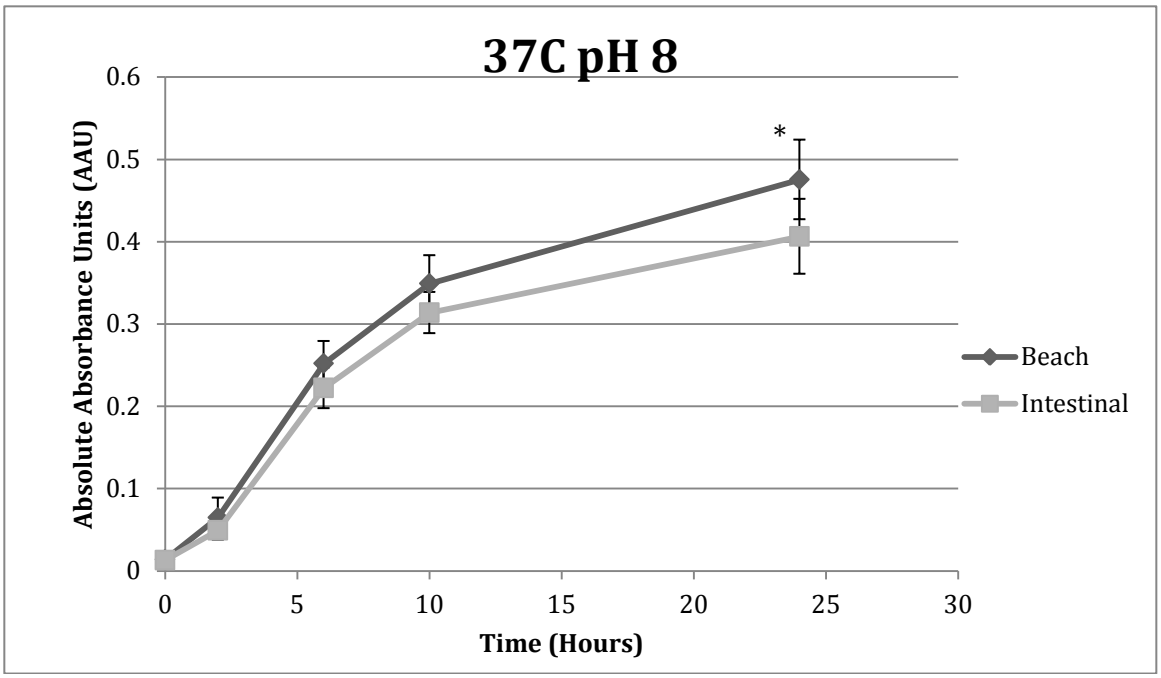

Figure 11. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 37˚C and cultured in Luria Broth adjusted to pH 8. Error bars indicate standard deviation.

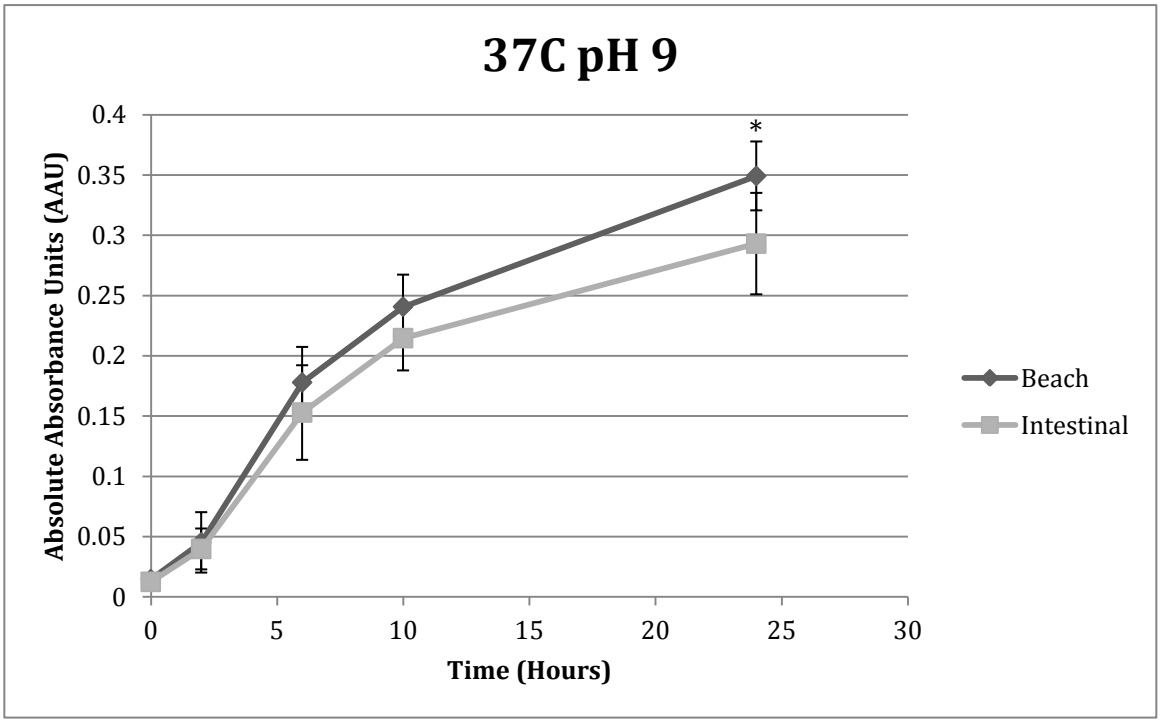

Figure 12. Mean absorbance of Beach and Intestinal *E. coli* isolates incubated at 37˚C and cultured in Luria Broth adjusted to pH 9. Error bars indicate standard deviation.

CHAPTER IV

DISCUSSION

Comparison of Carbon Source Use Between Intestinal and Beach *E. coli*

The primary (colon) and secondary (beach sand) habitats of *E. coli* differ greatly in terms of temperature, pH, UV exposure, oxygen content, moisture, and nutrient availability. From the perspective of bacterial growth, relatively speaking, soils and sediments are chronically nutrient deficient while the intestinal environment is endowed with nutrients (24), supporting hearty bacterial growth. For instance, with 70-100 g/day of dietary protein, the average concentration of amino acids in the human colon is approximately 100 μmole/L of intestinal contents, with 20-30% of amino acids available in free form (20). In contrast, the concentration of amino acids in sediment is 0.1 μmole/L with 0.5% available in free form (20, 41). A typical Western diet that generates ~100 g stool/day, is estimated to include at least 30 g of carbohydrate entering the colon. The profile and approximate percentages of carbohydrate types entering the colon are as follows: 50% nonstarch polysaccharide, 30-40% starch, 3-6% indigestible oligosaccharides, and 3-6% intestinal mucopolysaccharides (13). However, changes in diet can lead to fluctuations in the amount of carbohydrates, resulting in several fold increases or decreases in the amount of carbohydrates entering the colon. In spite of the high variability in types and concentrations of carbohydrates experienced by the colon microbial communities, intestinal communities are exposed to significantly greater abundance of carbohydrates than environmental communities. The vast majority of carbohydrates are found in nature as polysaccharides, with monosaccharides and

disaccharides accounting for only a small fraction of the total carbohydrates found in the extra-host environment (6).

With the relative high abundance and consistent presence of nutrients in the human colon compared to extra-intestinal environments, it might be expected that *E. coli,* which thrive in the secondary habitat, would exhibit a thriftier and more diverse metabolism (6) than *E. coli* residing in the primary habitat. In addition, it would be anticipated that if *E. coli* does adapt to a secondary habitat, adaptations would occur to environmental parameters that differ from its previous environment, such as differences in carbohydrate and amino acid availability. Based on the relative presence of amino acids and carbohydrates in the human colon versus beach sand, one could predict that Beach *E. coli* would more efficiently metabolize amino acids and carbohydrates than would Intestinal *E. coli*.

Interestingly, at the 6 hr incubation mark, the majority of the carbon sources more quickly metabolized by Beach *E. coli* were carbohydrates, while the majority of carbon sources more quickly metabolized by Intestinal *E. coli* were amino acids (Fig. 4). However, at the 24 hr incubation mark, with the exception of ρ-hydroxyphenylacetic acid, the statistical significance of the carbohydrate metabolic rates disappeared between the two isolate types. Thus, there appear to be selective pressures within the beach sand environment that may shift the efficiency of metabolism of Beach *E. coli* toward carbohydrates at the expense of overall amino acid metabolism.

The observed differences in carbon source utilization rates may speak to a competitive adaptation of Beach *E. coli* to the uniquely available carbon sources within the beach environment. Savageau (34) hypothesized that *E. coli,* in response to the transition from its primary into its secondary habitat, makes use of a dual regulation system. The

metabolic regulatory system Savageau proposed operates in response to the specific demands and available carbon sources of the primary and secondary environments; genes with products essential to that environment would be under positive control and genes with non-essential products would be under negative control. Espinosa-Urgel & Kolter (9) showed that certain genes coding for tRNA operons, *gapC*, and unknown functions in *E. coli* are preferentially expressed in the external environment (i.e. secondary habitat), suggesting that certain genes in *E. coli* play important roles in extra-host settings and may be under the control of a separate regulation system than genes important in the primary habitat. Genes associated with amino acid metabolism would be under positive control while *E. coli* were in the primary habitat and genes associated with carbohydrate metabolism would be under positive control while *E. coli* were present in the secondary habitat. Thus, in the colon with its high amino acid availability, genes in *E. coli* associated with amino acid metabolism under a positive control system, explaining the quicker metabolism of amino acids by Intestinal *E. coli* isolates versus Beach *E. coli*. Furthermore, it would be expected that the genes under positive regulation would be subject to selective pressure within the environment where their products are most important, which would account for the observed differences when comparing the amino acid metabolism and/or carbohydrate metabolism of Intestinal versus Beach *E. coli*.

Influence of UV light, in combination with Ambient Drying and Heat, on Subsequent Culturability of Intestinal versus Beach *E. coli*

Ultraviolet (UV) light is used as an effective method of microbial disinfection for water, air, and various surfaces. UV light induced inactivation of microorganisms is largely attributable to photobiochemical reactions, which occur within the microorganisms. The

majority of the UV light's germicidal range (200-300 nm) is composed of UV-B (280-320 nm) and UV-C (100-280 nm) wavelengths that produce pyrimidine dimers within DNA, in addition to other photoproducts (26). Furthermore, UV-A wavelengths (320-400 nm) produce reactive species, for example $O_2^{\bullet}$, H_2O_2, and $^{\bullet}OH$, which can subsequently damage genetic material and other intracellular molecules (32).

In response to the damaging effects of UV light, bacteria have evolved repair methods for UV-induced damage to cellular components. Among the best studied of the bacterial UV-induced DNA damage repair mechanisms are the photoreactiviation and dark-repair systems of *E. coli*; photoreactivation being a major player in repair of UV-induced damage, with only a relatively small contribution from dark repair mechanisms (14, 30). Photoreactivation results in the direct reversal of the UV damage by employing a photolyase that uses the energy of near-UV light (310-480 nm) to repair UV-induced lesions. Thus, even though UV-A can produce lethal and sublethal effects, indicated above, it is also essential to catalyze photoreactivation (26).

The available literature reports differences among strains of *E. coli* with respect to UV resistance and susceptibility. However, experimental results from this study, summarized in Table 3, show no significant difference ($p>0.05$) between post UV-exposure culturability of Intestinal and Beach *E. coli*. Furthermore, colony-forming units following sunlight exposure were low. *E. coli* entering a viable but non-culturable state, induced by insolation (11) may explain this observation. Another explanation for low culturability may be that the 20 minute exposure time to UV-A light was not sufficient for photoreactivation to take place, as one to three hours is typically required for the completion of photoreactivation repair (26). The photoreactivation repair mechanism is more reliant on

exposure time to photoreactive light than irradiance of the light, and the frequency of photolyase attachment to dimers is the limiting factor (16). Furthermore, if the UF-5 Plexiglas® blocked the specific wavelengths of UV-A utilized by photolyases, but did not block all damaging effects of UV-A, this would account for the low CFUs recovered in the Non-UV exposed groups.

With the conflict between the results generated in this study and the available literature, further experimentation is warranted to resolve the observed inconsistencies. One way to attain greater confidence in the results would be to use methods with higher specificity and sensitivity, in regards to selective growth and assessment of the state of organism viability. Doing so would subsequently increase the resolution of post UV-exposure viability of *E. coli*. Examples of such methods might include a culture-independent approach where mRNA, from a housekeeping gene, could be extracted from *E. coli* inoculated in sand, post UV-exposure, and quantified with real-time PCR to assess isolate viability. In addition, an endonuclease sensitive site assay (30) could be employed to assess the level of UV-induced damage to DNA in *E. coli* cells. These culture-independent methods would provide better resolution for observing differences or similarities in UV resistance/susceptibility between the two isolates.

Influence of pH and Temperature on Growth Rates of Intestinal versus Beach *E. coli*

Natural environments have been found to range in pH from approximately 0.5 to 10.5, with microbial growth present at every point within that range. In contrast, the mammalian intestinal environment has minimal pH deviation from 6.8 (25) and an accompanying narrow temperature range of 34.4˚C to 37.8˚C (36). It is generally accepted

that *E. coli* grow optimally at a pH of 6.8 and temperature of 37°C (25, 34), with a minimum and maximum pH tolerance for *E. coli* of 4.4 and 9.2, respectively (34, 28, 29). Yet, in this study Beach and Intestinal *E. coli* grew optimally at 19°C and pH 7 (Figure 6).

The life cycle of *E. coli* has been previously assumed to consist of hundreds to thousands of generations occurring within the primary habitat of the mammalian intestine, followed by brief stints enduring the elements of the natural environment that result in subsequent inoculation of a new host or cell death (34, 27). In regards to pH and temperature, the claim that adaptation evolves to match the degree of environmental variation (38, 10, 35) would imply that *E. coli* is be capable of surviving a range of pH and temperature conditions, but is best suited to the narrow range of the primary habitat. However, relatively recent studies (10, 3, 33) have found that *E. coli* do not simply endure the natural environment, but persist and even flourish. Taking these findings into consideration, one may predict, that in comparison, *E. coli* found in the natural environment may be more fit and/or tolerant to variable growth conditions (e.g. pH, temperature, etc.) than intestinal-isolated *E. coli*.

The results from the experiment that assessed the influence of pH and temperature on growth rate of the *E. coli* isolates demonstrated a statistically significant difference between the growth rates of Intestinal and Beach *E. coli* (Fig. 1 & 2). Beach *E. coli* grew more quickly and attained higher cell densities than Intestinal isolated *E. coli* at all pH and temperatures tested with the exception of one pH/temperature point. Interestingly, the exception was pH 6.8 and incubation temperature of 37°C, that is to say the conditions of human intestinal environment, and only in this instance was there no statistically significant difference between the growth rates of the two isolate types.

Significantly higher growth rates exhibited by Beach *E. coli,* at the vast majority of pH levels and temperatures tested, suggest that when compared to Intestinal *E. coli*, Beach *E. coli* have wider pH and temperature growth tolerances. Furthermore, it seems logical that a greater tolerance would impart a competitive advantage by allowing Beach *E. coli* to more readily adapt and persist under variable environmental conditions, relative to the homeostatic environment of the intestine.

The results of these studies support the environmental adaptation and naturalization model, whereby *E. coli*, when introduced into a beach environment, adapt and become naturalized to the environmental variability of the alternate habitat. In comparing carbon source use between Intestinal and Beach *E. coli*, the model would predict that *E. coli* would adapt to the types of carbon sources present in their respective environments. In agreement with the model, significant differences were shown in the metabolism of carbohydrates and amino acids; both isolate types demonstrated preference for the type of carbon source most prevalent in their respective environment. In regards to the influence of UV light, in combination with ambient drying and heat, on subsequent culturability of Intestinal versus Beach *E. coli*, the model would predict Beach *E. coli* to be more capable of coping with the combined stresses of UV exposure, ambient drying and heat. However, results from the experiment were not in agreement with the model, as no significant differences were found in the culturability of Beach or Intestinal *E. coli* post exposure. However, the available literature does indicate differences in UV resistance and susceptibility among strains of *E. coli*, which is why further studies employing more sensitive methods with better resolution are suggested. In terms of tolerance of pH and temperature, the model predicts that Intestinal *E. coli* would be less fit than Beach *E. coli* to

the wide range of pH and temperatures associated with the beach sand environment. Interestingly, results from the experiment confirmed the model's prediction in that Beach *E. coli* showed significantly higher growth rates at all pH and temperatures tested, with the exception of the combined pH 6.8 and incubation temperature of 37°C.

Alternatively, recent studies by Walk et al and Clermont et al, have suggested that cryptic lineages of *E. coli* may exist in the environment. If true, these cryptic *Escherchia* may potentially account for the observed differences in fitness between the two isolate types used in this study. Nonetheless, the utility of *E. coli* as an indicator organism for the presence of fecal contamination has been challenged by the results of this study and others (4, 18, 19), which have identified *E. coli* that are able to persist and grow under conditions of the extra-intestinal environment.

CHAPTER V

CONCLUSION

Significant differences were observed in carbon source utilization between Beach and Intestinal *E. coli*. Beach *E. coli* demonstrated higher metabolic activity with carbohydrates than Intestinal *E. coli* at the 6 hr incubation point. Intestinal *E. coli* had higher metabolic activity than Beach *E. coli* with amino acid carbon sources at both the 6 & 24 hr incubation time points. The observed differences in carbon source utilization rates may speak to a competitive adaptation of Beach *E. coli* to the uniquely available carbon sources within the beach environment.

This study could not demonstrate differences in the influence of UV light, in combination with ambient drying and heat, on the subsequent culturability of Intestinal versus Beach *E. coli*. There is the possibility that *E. coli* entered a viable but non-culturable state due to insolation or that the UF-5 Plexiglas® blocked wavelengths in the UV-A spectrum crucial to the operation of photolyases, while allowing passage of wavelengths resulting in lethal effects on *E. coli*. Further studies with more sensitive culture-independent methods should be employed, such as extracting mRNA of a housekeeping gene, from *E. coli* inoculated in sand, post UV-exposure, and quantifying with real-time PCR to assess isolate viability. In addition, an endonuclease sensitive site assay could be employed to assess the level of UV-induced damage to DNA in *E. coli* cells.

Beach *E. coli* grew more quickly and attained higher cell densities than Intestinal isolated *E. coli* at all pH and temperatures tested with the exception of one pH/temperature point. There was no statistically significant difference between the two isolates' growth

rates at pH 6.8 and incubation temperature of 37°C, which are the conditions of the human intestinal environment. Significantly higher growth rates exhibited by Beach *E. coli*, at the vast majority of pH levels and temperatures tested, suggest that when compared to Intestinal *E. coli*, Beach *E. coli* have wider pH and temperature growth tolerances.

The results of these studies suggest that relative to Intestinal *E. coli*, Beach *E. coli* demonstrate increased fitness to the environmental conditions found at freshwater beaches of the Great Lakes. Thus, these studies support the environmental adaptation and naturalization model, whereby *E. coli*, when introduced into a beach environment, adapt and become naturalized to the environmental variability of the alternate habitat.

The importance of these findings are realized bearing in mind that *E. coli*'s inherent association with the intestinal environment has led to its use as a fecal indicator organism; if *E. coli* persists and adapts to the beach environment, then its use as a fecal indicator organism is compromised. Furthermore, the mechanisms by which *E. coli* survives in and/or adapts to the beach sand environment are poorly understood and this realization undoubtedly provides incentive for further investigation.

APPENDICES

APPENDIX A

BIOLOG RAW DATA FOR INTESTINAL *E. COLI* ISOLATES (1-5) AT 6 HR INCUBATION POINT.

	Isolate Type				
Carbon Source	**I(1)**	**I(2)**	**I(3)**	**I(4)**	**I(5)**
Water	0	0	0	0	0
α-Cyclodextrin	16	6	3	7	12
Dextrin	191	89	89	75	157
Glycogen	11	12	7	5	16
Tween 40	14	10	8	4	14
Tween 80	19	7	7	11	13
N-Acetyl-D-Galactosamine	19	186	180	80	144
N-Acetyl-D-Glucosamine	322	307	327	90	235
Adonitol	18	10	6	7	12
L-Arabinose	288	338	252	130	260
D-Arabitol	10	0	4	0	6
D-Cellobiose	23	7	1	14	16
i-Erythritol	1	4	-8	2	12
D-Fructose	288	358	284	97	158
L-Fucose	98	184	128	34	118
D-Galactose	54	173	58	98	132
Gentiobiose	12	0	0	6	4
α-D-Glucose	307	375	304	94	135
m-inositol	22	12	3	7	16
α-D-Lactose	136	211	198	74	236
Lactulose	6	6	6	13	12
Maltose	155	224	178	121	230
D-Mannitol	305	330	276	72	237
D-Mannose	324	338	285	127	281
D-Meliblose	8	10	170	113	73
β-Methyl-D-Glucoside	48	24	21	48	39
D-Psicose	120	125	144	129	146
D-Raffinose	-1	1	-8	1	17
L-Rhamnose	6	200	121	-2	37
D-Sorbitol	72	180	120	135	240
Sucrose	14	121	6	11	187
D-Trehalose	281	289	231	144	287
Turanose	16	8	5	13	15
Xylitol	2	4	-10	-2	10
Pyruvic Acid Methyl Ester	99	129	65	44	59

Succinic Acid Mono-Methyl Ester	12	88	14	15	22
Acetic Acid	33	91	61	84	58
Cis-Aconitic Acid	4	-5	-12	8	14
Citric Acid	6	9	4	4	11
Formic Acid	3	1	-2	4	14
D-Glatonic Acid Lactone	158	16	31	20	22
D-Glacturonic Acid	167	260	126	16	179
D-Gluconic Acid	202	161	217	69	160
D-Glucosaminic Acid	10	4	-3	7	15
D-Glucuronic Acid	179	39	166	88	208
α-Hydroxybutyric Acid	36	14	33	67	25
β-Hydroxybutyric Acid	11	15	1	3	13
γ-Hydroxybutyric Acid	10	7	-5	8	5
ρ-Hydroxy Phenylacetic Acid	15	5	0	2	10
Itaconic Acid	6	-2	-13	6	13
α-Ketobutyric Acid	-43	4	7	12	17
α-Ketoglutaric Acid	7	3	2	4	15
α-Ketovaleric Acid	15	10	6	2	16
D,L-Lactic Acid	24	132	90	156	138
Malonic Acid	8	-78	9	9	10
Propionic Acid	31	22	7	15	31
Quinic Acid	8	1	3	9	6
D-Saccharic Acid	17	14	8	12	19
Sebacic Acid	16	2	1	4	16
Succinic Acid	26	118	77	81	130
Bromosuccinic Acid	40	31	30	64	47
Succinamic Acid	22	6	-1	6	15
Glucuronamide	134	122	153	20	181
L-Alaninamide	9	16	0	11	17
D-Alanine	27	13	2	9	19
L-Alanine	55	162	5	88	79
L-Alanyl-glycine	58	116	-2	69	90
L-Asparagine	40	140	86	100	107
L-Aspartic Acid	24	107	49	64	37
L-Glutamic Acid	14	12	4	7	16
Glycyl-L-Aspartic Acid	77	125	57	75	200
Glycyl-L-Glutamic Acid	16	14	16	10	26
L-Histidine	13	-4	-1	2	3

Hydroxy-L-Proline	18	8	0	5	12
L-Leucine	19	3	13	10	22
L-Ornithine	12	-1	0	-5	13
L-Phenylalanine	14	6	3	7	15
L-Proline	8	3	12	8	14
L-Pyroglutamic Acid	9	3	-1	2	11
D-Serine	149	139	83	23	152
L-Serine	68	149	59	73	120
L-Threonine	20	9	7	16	20
d,L-Carnitine	6	10	-2	-5	16
γ-Aminobutyric Acid	18	3	0	9	10
Urocanic Acid	15	3	8	2	13
Inosine	248	222	201	92	220
Uridine	227	326	226	110	274
Thymidine	314	234	168	161	256
Phenylethylamine	19	11	3	11	15
Putrescine	18	14	7	12	20
2-Aminoethanol	9	3	0	0	8
2,3-Butanediol	12	7	8	0	15
Glycerol	50	221	52	48	97
D,L-α-Glycerol Phosphate	59	108	68	105	134
α-D-Glucose-1-Phosphate	303	342	295	152	272
D-Glucose-6-Phosphate	316	320	273	99	281

APPENDIX B

BIOLOG RAW DATA FOR INTESTINAL *E. COLI* ISOLATES (6-10) AT 6 HR INCUBATION POINT.

	Isolate Type				
Carbon Source	**I(6)**	**I(7)**	**I(8)**	**I(9)**	**I(10)**
Water	0	0	0	0	0
α-Cyclodextrin	4	-1	2	8	1
Dextrin	95	108	52	101	35
Glycogen	6	8	4	6	-2
Tween 40	6	3	8	12	4
Tween 80	1	1	6	9	87
N-Acetyl-D-Galactosamine	192	131	90	58	310
N-Acetyl-D-Glucosamine	250	290	157	82	232
Adonitol	4	1	6	11	9
L-Arabinose	137	233	26	172	217
D-Arabitol	3	-2	0	3	1
D-Cellobiose	10	9	10	25	1
i-Erythritol	3	-11	6	2	2
D-Fructose	202	231	171	70	306
L-Fucose	41	76	62	17	84
D-Galactose	83	134	74	24	34
Gentiobiose	8	-1	0	3	5
α-D-Glucose	209	224	174	75	308
m-inositol	8	4	0	7	109
α-D-Lactose	181	219	143	59	75
Lactulose	9	3	-1	8	-3
Maltose	170	200	65	72	-6
D-Mannitol	170	188	140	70	128
D-Mannose	116	308	213	97	254
D-Meliblose	207	149	5	78	34
β-Methyl-D-Glucoside	79	42	67	26	24
D-Psicose	156	159	118	158	91
D-Raffinose	9	-1	-8	9	3
L-Rhamnose	108	104	9	6	76
D-Sorbitol	127	139	6	65	338
Sucrose	10	5	1	9	366
D-Trehalose	176	239	179	90	114
Turanose	14	5	2	11	5
Xylitol	-2	-3	2	5	-9
Pyruvic Acid Methyl Ester	53	50	36	48	60

Succinic Acid Mono-Methyl Ester	20	18	22	83	85
Acetic Acid	80	68	6	86	66
Cis-Aconitic Acid	6	-3	19	11	0
Citric Acid	-2	-4	-3	5	7
Formic Acid	51	6	7	61	-5
D-Glatonic Acid Lactone	8	21	14	30	11
D-Glacturonic Acid	174	163	86	98	203
D-Gluconic Acid	132	127	91	49	119
D-Glucosaminic Acid	4	2	1	3	-2
D-Glucuronic Acid	192	115	97	131	146
α-Hydroxybutyric Acid	24	20	16	91	34
β-Hydroxybutyric Acid	5	-4	1	14	2
γ-Hydroxybutyric Acid	-7	3	8	5	-2
ρ-Hydroxy Phenylacetic Acid	4	5	2	3	-2
Itaconic Acid	-1	2	-1	2	6
α-Ketobutyric Acid	8	7	11	47	13
α-Ketoglutaric Acid	6	6	-1	3	-8
α-Ketovaleric Acid	7	8	-3	14	6
D,L-Lactic Acid	86	118	87	134	60
Malonic Acid	1	4	4	7	-4
Propionic Acid	0	8	18	52	77
Quinic Acid	5	1	1	-2	-5
D-Saccharic Acid	2	3	7	12	11
Sebacic Acid	3	7	-1	8	3
Succinic Acid	93	115	93	109	115
Bromosuccinic Acid	91	84	61	126	86
Succinamic Acid	6	8	6	9	5
Glucuronamide	173	169	25	137	135
L-Alaninamide	15	2	-5	16	14
D-Alanine	15	4	3	32	6
L-Alanine	99	61	81	106	102
L-Alanyl-glycine	134	124	111	102	129
L-Asparagine	93	151	116	131	158
L-Aspartic Acid	110	97	104	145	148
L-Glutamic Acid	11	3	4	14	14
Glycyl-L-Aspartic Acid	82	262	69	141	138
Glycyl-L-Glutamic Acid	17	30	15	23	23
L-Histidine	2	0	1	1	5

Hydroxy-L-Proline	-1	5	0	1	2
L-Leucine	0	8	14	7	17
L-Ornithine	6	-5	-1	0	5
L-Phenylalanine	2	4	1	9	5
L-Proline	3	2	5	8	5
L-Pyroglutamic Acid	7	0	12	4	-4
D-Serine	62	121	57	30	37
L-Serine	77	129	81	112	82
L-Threonine	6	11	14	17	6
d,L-Carnitine	2	7	-5	2	7
γ-Aminobutyric Acid	2	1	1	1	3
Urocanic Acid	2	3	3	2	-4
Inosine	83	136	80	106	38
Uridine	187	258	183	98	164
Thymidine	220	259	207	109	213
Phenylethylamine	-1	9	6	8	3
Putrescine	7	7	6	7	-5
2-Aminoethanol	9	3	0	0	8
2,3-Butanediol	12	7	8	0	15
Glycerol	50	221	52	48	97
D,L-α-Glycerol Phosphate	59	108	68	105	134
α-D-Glucose-1-Phosphate	303	342	295	152	272
D-Glucose-6-Phosphate	316	320	273	99	281

APPENDIX C

BIOLOG RAW DATA FOR BEACH *E. COLI* ISOLATES (1-5) AT 6 HR INCUBATION POINT.

	Isolate Type				
Carbon Source	**B(1)**	**B(2)**	**B(3)**	**B(4)**	**B(5)**
Water	0	0	0	0	0
α-Cyclodextrin	17	1	-5	17	13
Dextrin	165	49	95	131	163
Glycogen	16	7	3	19	23
Tween 40	8	5	10	32	10
Tween 80	22	13	1	75	17
N-Acetyl-D-Galactosamine	162	76	181	415	178
N-Acetyl-D-Glucosamine	179	198	235	587	355
Adonitol	11	9	-5	20	5
L-Arabinose	347	268	305	378	308
D-Arabitol	5	6	-11	21	11
D-Cellobiose	15	18	6	16	19
i-Erythritol	10	9	-7	5	8
D-Fructose	172	182	226	447	361
L-Fucose	142	100	119	316	112
D-Galactose	89	25	62	163	70
Gentiobiose	11	4	-1	17	6
α-D-Glucose	194	174	226	400	310
m-inositol	18	9	-2	17	23
α-D-Lactose	121	161	117	337	204
Lactulose	11	8	0	11	15
Maltose	167	133	149	280	186
D-Mannitol	160	278	266	440	215
D-Mannose	290	266	243	453	312
D-Meliblose	180	127	172	342	158
β-Methyl-D-Glucoside	61	15	48	30	20
D-Psicose	123	72	102	159	133
D-Raffinose	36	10	7	34	17
L-Rhamnose	134	88	145	310	127
D-Sorbitol	163	101	216	263	151
Sucrose	17	17	5	21	17
D-Trehalose	237	118	205	320	305
Turanose	15	11	3	15	15
Xylitol	5	5	-7	2	9
Pyruvic Acid Methyl Ester	76	55	80	314	97

Succinic Acid Mono-Methyl Ester	20	8	5	92	51
Acetic Acid	55	19	53	61	62
Cis-Aconitic Acid	14	8	-5	4	4
Citric Acid	11	10	2	8	15
Formic Acid	14	24	-5	11	50
D-Glatonic Acid Lactone	86	66	34	380	26
D-Glacturonic Acid	97	161	180	517	168
D-Gluconic Acid	151	221	212	582	175
D-Glucosaminic Acid	5	23	1	16	11
D-Glucuronic Acid	210	61	181	426	182
α-Hydroxybutyric Acid	61	32	53	59	75
β-Hydroxybutyric Acid	25	12	-5	10	17
γ-Hydroxybutyric Acid	11	9	-6	7	10
ρ-Hydroxy Phenylacetic Acid	96	73	68	73	102
Itaconic Acid	8	6	-3	3	16
α-Ketobutyric Acid	17	18	34	20	60
α-Ketoglutaric Acid	15	4	1	8	13
α-Ketovaleric Acid	17	21	1	14	14
D,L-Lactic Acid	83	96	37	114	101
Malonic Acid	12	14	1	17	5
Propionic Acid	18	25	14	72	19
Quinic Acid	15	13	-19	6	1
D-Saccharic Acid	17	17	3	23	12
Sebacic Acid	7	12	-5	2	19
Succinic Acid	34	13	32	98	110
Bromosuccinic Acid	30	23	18	58	88
Succinamic Acid	14	12	-2	22	14
Glucuronamide	150	49	102	142	79
L-Alaninamide	19	19	-5	12	17
D-Alanine	17	3	10	69	26
L-Alanine	103	11	65	116	94
L-Alanyl-glycine	88	26	36	83	126
L-Asparagine	117	8	101	37	136
L-Aspartic Acid	103	24	29	64	167
L-Glutamic Acid	22	4	5	27	17
Glycyl-L-Aspartic Acid	76	42	76	100	118
Glycyl-L-Glutamic Acid	5	16	14	28	23
L-Histidine	7	5	-6	5	2

Hydroxy-L-Proline	5	1	6	12	12
L-Leucine	22	7	4	20	27
L-Ornithine	9	0	3	20	15
L-Phenylalanine	17	2	-4	11	1
L-Proline	19	19	-1	19	14
L-Pyroglutamic Acid	10	10	-22	14	3
D-Serine	21	5	0	15	15
L-Serine	33	9	10	46	59
L-Threonine	25	10	3	28	26
d,L-Carnitine	17	7	0	21	12
γ-Aminobutyric Acid	16	5	4	10	14
Urocanic Acid	12	8	4	16	8
Inosine	276	206	176	423	180
Uridine	267	82	226	364	327
Thymidine	182	179	243	391	311
Phenylethylamine	16	12	4	10	11
Putrescine	6	17	-16	20	18
2-Aminoethanol	9	14	-19	14	9
2,3-Butanediol	8	16	7	13	12
Glycerol	130	31	46	119	105
D,L-α-Glycerol Phosphate	55	74	90	90	80
α-D-Glucose-1-Phosphate	295	271	301	658	351
D-Glucose-6-Phosphate	226	258	266	699	269

APPENDIX D

BIOLOG RAW DATA FOR BEACH *E. COLI* ISOLATES (6-10) AT 6 HR INCUBATION POINT.

	Isolate Type				
Carbon Source	**B(6)**	**B(7)**	**B(8)**	**B(9)**	**B(10)**
Water	0	0	0	0	0
α-Cyclodextrin	14	5	9	1	7
Dextrin	106	180	65	79	135
Glycogen	11	12	20	16	19
Tween 40	17	2	11	-7	5
Tween 80	15	5	19	4	5
N-Acetyl-D-Galactosamine	38	149	89	183	118
N-Acetyl-D-Glucosamine	262	217	250	305	180
Adonitol	-1	5	9	6	14
L-Arabinose	238	373	324	246	330
D-Arabitol	11	1	10	15	12
D-Cellobiose	17	4	15	34	10
i-Erythritol	10	4	10	-6	-4
D-Fructose	221	170	204	270	221
L-Fucose	87	180	117	132	147
D-Galactose	43	79	63	46	123
Gentiobiose	2	2	5	12	14
α-D-Glucose	315	168	189	319	180
m-inositol	9	7	13	10	0
α-D-Lactose	144	151	127	160	151
Lactulose	2	2	7	16	18
Maltose	153	155	151	189	172
D-Mannitol	239	260	288	211	245
D-Mannose	302	273	309	235	217
D-Meliblose	144	161	138	193	136
β-Methyl-D-Glucoside	25	73	40	59	23
D-Psicose	88	101	107	107	69
D-Raffinose	7	6	14	80	15
L-Rhamnose	41	160	150	161	129
D-Sorbitol	122	227	135	81	126
Sucrose	-7	12	11	9	2
D-Trehalose	169	240	192	165	206
Turanose	7	8	13	21	10
Xylitol	6	2	11	-6	-1
Pyruvic Acid Methyl Ester	57	89	86	100	78

Succinic Acid Mono-Methyl Ester	12	4	19	5	13
Acetic Acid	17	32	24	33	7
Cis-Aconitic Acid	4	-1	10	0	-9
Citric Acid	5	1	10	2	-11
Formic Acid	0	-1	9	-1	1
D-Glatonic Acid Lactone	6	99	39	165	76
D-Glacturonic Acid	-12	110	67	177	170
D-Gluconic Acid	167	195	255	197	206
D-Glucosaminic Acid	6	6	4	7	6
D-Glucuronic Acid	12	102	29	140	97
α-Hydroxybutyric Acid	22	64	33	37	6
β-Hydroxybutyric Acid	22	5	12	1	3
γ-Hydroxybutyric Acid	9	7	11	5	-2
ρ-Hydroxy Phenylacetic Acid	22	46	7	-2	21
Itaconic Acid	5	3	4	-5	-7
α-Ketobutyric Acid	13	26	14	27	4
α-Ketoglutaric Acid	5	-3	-1	2	6
α-Ketovaleric Acid	6	16	18	7	4
D,L-Lactic Acid	83	58	82	65	55
Malonic Acid	9	5	-57	0	4
Propionic Acid	7	24	17	19	13
Quinic Acid	6	-8	2	0	-2
D-Saccharic Acid	26	3	6	-1	-2
Sebacic Acid	14	-5	17	-2	-4
Succinic Acid	9	46	15	23	0
Bromosuccinic Acid	3	10	18	31	10
Succinamic Acid	2	14	17	14	-1
Glucuronamide	7	89	21	92	113
L-Alaninamide	8	11	12	14	3
D-Alanine	2	15	17	7	3
L-Alanine	0	100	22	55	59
L-Alanyl-glycine	8	90	22	19	70
L-Asparagine	7	97	21	107	26
L-Aspartic Acid	12	27	22	46	14
L-Glutamic Acid	6	8	20	19	8
Glycyl-L-Aspartic Acid	48	64	75	67	47
Glycyl-L-Glutamic Acid	3	8	15	10	16
L-Histidine	0	5	9	9	-10

Hydroxy-L-Proline	-1	6	19	2	-10
L-Leucine	10	3	15	1	-3
L-Ornithine	0	8	3	1	3
L-Phenylalanine	6	0	12	-1	-8
L-Proline	2	10	14	8	4
L-Pyroglutamic Acid	6	-4	8	6	0
D-Serine	-1	14	5	11	4
L-Serine	4	24	11	13	20
L-Threonine	14	14	11	11	19
d,L-Carnitine	-3	-61	24	1	1
γ-Aminobutyric Acid	5	8	19	10	-3
Urocanic Acid	21	12	6	-5	3
Inosine	214	235	235	90	154
Uridine	116	206	239	159	196
Thymidine	195	184	236	157	170
Phenylethylamine	11	6	16	4	5
Putrescine	17	-3	15	0	1
2-Aminoethanol	2	-6	14	9	-1
2,3-Butanediol	16	8	15	5	5
Glycerol	42	69	43	12	47
D,L-α-Glycerol Phosphate	84	93	84	35	59
α-D-Glucose-1-Phosphate	285	284	317	251	177
D-Glucose-6-Phosphate	302	259	272	227	202

APPENDIX E

BIOLOG RAW DATA FOR INTESTINAL *E. COLI* ISOLATES (1-5) AT 24 HR INCUBATION POINT.

	Isolate Type				
Carbon Source	**I(1)**	**I(2)**	**I(3)**	**I(4)**	**I(5)**
Water	0	0	0	0	0
α-Cyclodextrin	17	5	1	16	21
Dextrin	261	86	106	89	222
Glycogen	18	13	11	15	21
Tween 40	13	16	14	18	27
Tween 80	34	15	14	29	20
N-Acetyl-D-Galactosamine	13	356	364	140	407
N-Acetyl-D-Glucosamine	710	624	641	242	672
Adonitol	25	15	1	21	16
L-Arabinose	693	705	692	577	862
D-Arabitol	6	2	-1	1	5
D-Cellobiose	19	5	32	13	20
i-Erythritol	-6	-5	-11	6	17
D-Fructose	548	656	573	212	322
L-Fucose	294	416	378	128	279
D-Galactose	96	360	203	143	350
Gentiobiose	22	5	12	17	13
α-D-Glucose	540	670	576	194	329
m-inositol	34	18	17	26	29
α-D-Lactose	309	444	464	157	439
Lactulose	148	105	98	51	168
Maltose	390	462	458	185	443
D-Mannitol	536	723	522	133	467
D-Mannose	604	649	539	187	470
D-Meliblose	110	172	359	106	231
β-Methyl-D-Glucoside	163	14	50	148	248
D-Psicose	202	156	187	162	181
D-Raffinose	9	17	1	7	103
L-Rhamnose	27	417	350	17	254
D-Sorbitol	220	372	256	207	449
Sucrose	31	385	24	29	386
D-Trehalose	556	623	543	306	503
Turanose	32	21	40	41	40
Xylitol	7	6	-10	7	15
Pyruvic Acid Methyl Ester	172	227	169	124	191

Succinic Acid Mono-Methyl Ester	20	149	34	41	90
Acetic Acid	61	66	96	82	112
Cis-Aconitic Acid	12	-2	-2	15	23
Citric Acid	10	14	11	19	19
Formic Acid	12	5	7	16	28
D-Glatonic Acid Lactone	383	96	138	69	222
D-Glacturonic Acid	528	792	493	115	850
D-Gluconic Acid	617	424	569	355	625
D-Glucosaminic Acid	22	13	11	27	27
D-Glucuronic Acid	497	511	625	157	864
α-Hydroxybutyric Acid	73	17	23	97	61
β-Hydroxybutyric Acid	21	19	10	17	25
γ-Hydroxybutyric Acid	9	6	-7	33	4
ρ-Hydroxy Phenylacetic Acid	9	4	-11	38	7
Itaconic Acid	13	0	-8	15	17
α-Ketobutyric Acid	-40	7	39	-5	34
α-Ketoglutaric Acid	17	11	14	21	24
α-Ketovaleric Acid	28	17	13	17	27
D,L-Lactic Acid	240	316	258	348	300
Malonic Acid	15	21	15	20	17
Propionic Acid	77	32	-7	10	83
Quinic Acid	19	6	14	27	15
D-Saccharic Acid	31	327	107	28	31
Sebacic Acid	23	4	6	13	23
Succinic Acid	23	141	98	121	188
Bromosuccinic Acid	127	43	38	93	49
Succinamic Acid	26	6	-5	16	28
Glucuronamide	351	223	390	182	393
L-Alaninamide	13	18	8	29	33
D-Alanine	42	24	11	22	31
L-Alanine	135	239	20	143	165
L-Alanyl-glycine	89	198	14	131	209
L-Asparagine	103	240	175	199	280
L-Aspartic Acid	91	165	81	124	84
L-Glutamic Acid	18	25	26	34	37
Glycyl-L-Aspartic Acid	108	237	195	152	405
Glycyl-L-Glutamic Acid	5	20	35	26	37
L-Histidine	9	-8	-5	6	-3

Hydroxy-L-Proline	23	12	0	13	24
L-Leucine	23	7	12	24	21
L-Ornithine	19	3	7	5	22
L-Phenylalanine	24	13	11	23	27
L-Proline	15	8	25	30	27
L-Pyroglutamic Acid	13	3	5	12	21
D-Serine	412	375	387	314	596
L-Serine	116	472	118	255	423
L-Threonine	53	15	46	58	46
d,L-Carnitine	12	11	-4	7	26
γ-Aminobutyric Acid	20	-2	7	14	10
Urocanic Acid	2	4	13	11	13
Inosine	494	440	544	244	429
Uridine	448	581	550	251	508
Thymidine	586	339	355	338	508
Phenylethylamine	21	11	11	26	25
Putrescine	22	13	13	25	21
2-Aminoethanol	11	4	1	6	9
2,3-Butanediol	15	6	12	5	24
Glycerol	190	313	213	168	224
D,L-α-Glycerol Phosphate	198	165	209	212	307
α-D-Glucose-1-Phosphate	675	649	562	341	582
D-Glucose-6-Phosphate	625	617	605	359	546

APPENDIX F

BIOLOG raw data for Intestinal *E. coli* isolates (6-10) at 24 hr incubation point.

	Isolate Type				
Carbon Source	**I(6)**	**I(7)**	**I(8)**	**I(9)**	**I(10)**
Water	0	0	0	0	0
α-Cyclodextrin	15	-3	5	21	23
Dextrin	270	132	64	187	58
Glycogen	21	8	7	20	22
Tween 40	38	5	13	34	32
Tween 80	135	6	14	33	96
N-Acetyl-D-Galactosamine	845	291	300	108	342
N-Acetyl-D-Glucosamine	874	696	383	366	694
Adonitol	17	5	12	32	44
L-Arabinose	660	807	454	521	561
D-Arabitol	-1	-13	-6	11	26
D-Cellobiose	19	19	22	36	15
i-Erythritol	1	-24	-5	4	8
D-Fructose	551	468	382	154	782
L-Fucose	435	280	215	65	274
D-Galactose	473	365	232	65	172
Gentiobiose	30	4	11	16	41
α-D-Glucose	594	493	429	214	638
m-inositol	27	23	18	30	44
α-D-Lactose	718	482	388	181	366
Lactulose	127	56	64	67	137
Maltose	687	456	244	139	30
D-Mannitol	557	536	342	201	441
D-Mannose	675	643	435	183	672
D-Meliblose	624	257	192	71	301
β-Methyl-D-Glucoside	292	145	150	127	155
D-Psicose	281	203	198	252	187
D-Raffinose	29	2	4	28	142
L-Rhamnose	539	287	185	40	343
D-Sorbitol	573	248	112	94	239
Sucrose	30	24	11	32	441
D-Trehalose	681	524	404	242	336
Turanose	79	39	22	41	49
Xylitol	2	0	-1	21	13
Pyruvic Acid Methyl Ester	339	209	128	164	238
Succinic Acid Mono-Methyl Ester	21	35	89	133	167

Acetic Acid	106	72	12	109	106
Cis-Aconitic Acid	23	-1	23	30	21
Citric Acid	14	-1	2	24	22
Formic Acid	72	5	15	54	17
D-Glatonic Acid Lactone	369	130	162	67	168
D-Glacturonic Acid	767	557	529	182	706
D-Gluconic Acid	698	424	469	340	471
D-Glucosaminic Acid	25	11	11	22	31
D-Glucuronic Acid	815	478	370	261	610
α-Hydroxybutyric Acid	41	22	38	137	75
β-Hydroxybutyric Acid	18	-1	-1	34	32
γ-Hydroxybutyric Acid	8	3	4	26	20
ρ-Hydroxy Phenylacetic Acid	7	-6	-6	23	15
Itaconic Acid	10	1	3	14	25
α-Ketobutyric Acid	23	71	27	-14	-18
α-Ketoglutaric Acid	38	11	11	22	18
α-Ketovaleric Acid	17	12	9	35	34
D,L-Lactic Acid	458	292	241	243	432
Malonic Acid	19	7	7	24	23
Propionic Acid	25	60	56	41	69
Quinic Acid	22	-5	7	13	16
D-Saccharic Acid	24	19	34	35	60
Sebacic Acid	19	4	1	20	27
Succinic Acid	161	169	150	171	259
Bromosuccinic Acid	124	90	68	179	89
Succinamic Acid	19	13	12	14	24
Glucuronamide	478	385	184	236	363
L-Alaninamide	129	11	14	40	38
D-Alanine	47	10	12	59	36
L-Alanine	135	187	209	209	171
L-Alanyl-glycine	244	181	184	178	246
L-Asparagine	176	256	312	220	243
L-Aspartic Acid	238	125	225	226	227
L-Glutamic Acid	92	16	14	39	45
Glycyl-L-Aspartic Acid	458	831	241	300	397
Glycyl-L-Glutamic Acid	69	43	12	49	30
L-Histidine	4	-5	-11	1	16
Hydroxy-L-Proline	21	4	9	8	22
L-Leucine	9	6	19	20	39

L-Ornithine	22	-5	4	6	29
L-Phenylalanine	12	3	8	23	24
L-Proline	24	6	19	30	29
L-Pyroglutamic Acid	17	-3	12	15	13
D-Serine	540	417	289	214	294
L-Serine	434	429	343	320	329
L-Threonine	26	38	25	55	40
d,L-Carnitine	21	9	-5	13	29
γ-Aminobutyric Acid	3	-2	0	3	24
Urocanic Acid	0	-2	-9	4	3
Inosine	500	343	242	231	218
Uridine	631	517	425	192	430
Thymidine	793	548	520	260	660
Phenylethylamine	7	13	10	17	18
Putrescine	9	1	10	24	14
2-Aminoethanol	10	-12	-8	22	9
2,3-Butanediol	8	-10	1	20	13
Glycerol	326	215	230	77	213
D,L-α-Glycerol Phosphate	332	234	208	170	200
α-D-Glucose-1-Phosphate	487	417	499	255	711
D-Glucose-6-Phosphate	604	642	400	435	755

APPENDIX G

BIOLOG RAW DATA FOR BEACH *E. COLI* ISOLATES (1-5) AT 24 HR INCUBATION POINT.

	Isolate Type				
Carbon Source	B(1)	B(2)	B(3)	B(4)	B(5)
Water	0	0	0	0	0
α-Cyclodextrin	19	3	-2	42	22
Dextrin	210	69	96	165	228
Glycogen	20	7	1	45	34
Tween 40	14	8	12	54	21
Tween 80	33	13	1	143	33
N-Acetyl-D-Galactosamine	346	174	310	973	363
N-Acetyl-D-Glucosamine	430	389	459	948	729
Adonitol	18	6	1	40	19
L-Arabinose	711	747	685	860	693
D-Arabitol	2	5	-14	39	23
D-Cellobiose	18	13	8	13	26
i-Erythritol	9	9	-18	5	7
D-Fructose	364	389	415	717	590
L-Fucose	278	266	232	773	142
D-Galactose	237	79	162	519	197
Gentiobiose	22	1	3	44	21
α-D-Glucose	400	334	411	636	537
m-inositol	28	19	15	38	37
α-D-Lactose	304	397	258	756	393
Lactulose	130	22	128	185	86
Maltose	326	339	279	713	349
D-Mannitol	360	546	457	629	388
D-Mannose	555	516	449	841	522
D-Meliblose	342	307	345	783	163
β-Methyl-D-Glucoside	93	46	109	199	97
D-Psicose	165	72	111	276	200
D-Raffinose	108	18	83	429	96
L-Rhamnose	256	243	292	916	247
D-Sorbitol	295	159	360	755	225
Sucrose	54	17	18	397	114
D-Trehalose	482	349	423	801	511
Turanose	36	16	25	41	50
Xylitol	8	7	-11	8	19
Pyruvic Acid Methyl Ester	178	130	169	530	259

Succinic Acid Mono-Methyl Ester	104	6	52	99	79
Acetic Acid	98	41	53	123	116
Cis-Aconitic Acid	22	10	1	26	17
Citric Acid	22	11	11	32	26
Formic Acid	21	22	-3	36	62
D-Glatonic Acid Lactone	136	176	117	937	82
D-Glacturonic Acid	410	489	445	984	242
D-Gluconic Acid	462	534	442	965	462
D-Glucosaminic Acid	16	25	14	47	21
D-Glucuronic Acid	616	263	463	906	233
α-Hydroxybutyric Acid	36	57	14	19	84
β-Hydroxybutyric Acid	30	15	0	72	34
γ-Hydroxybutyric Acid	16	6	-4	14	21
ρ-Hydroxy Phenylacetic Acid	140	107	88	271	137
Itaconic Acid	11	9	-4	20	27
α-Ketobutyric Acid	44	35	47	47	81
α-Ketoglutaric Acid	23	6	3	40	26
α-Ketovaleric Acid	19	29	11	23	29
D,L-Lactic Acid	215	201	150	420	282
Malonic Acid	17	17	7	42	19
Propionic Acid	49	46	49	49	73
Quinic Acid	25	16	-16	25	11
D-Saccharic Acid	26	25	207	533	30
Sebacic Acid	14	13	-7	17	34
Succinic Acid	110	15	62	176	149
Bromosuccinic Acid	45	18	37	235	101
Succinamic Acid	17	14	0	35	19
Glucuronamide	270	125	220	403	170
L-Alaninamide	30	23	10	51	34
D-Alanine	32	1	19	163	36
L-Alanine	111	24	49	254	151
L-Alanyl-glycine	73	82	2	366	128
L-Asparagine	210	17	126	123	232
L-Aspartic Acid	170	26	125	141	255
L-Glutamic Acid	43	5	16	79	37
Glycyl-L-Aspartic Acid	134	86	2	185	299
Glycyl-L-Glutamic Acid	32	13	30	59	43
L-Histidine	8	4	-11	3	0

Hydroxy-L-Proline	12	2	8	29	24
L-Leucine	36	8	3	48	32
L-Ornithine	13	1	11	57	26
L-Phenylalanine	25	1	1	31	12
L-Proline	33	16	7	47	25
L-Pyroglutamic Acid	18	13	-24	26	10
D-Serine	30	9	1	53	24
L-Serine	91	8	50	232	78
L-Threonine	67	20	33	52	106
d,L-Carnitine	27	8	2	34	25
γ-Aminobutyric Acid	25	0	2	24	23
Urocanic Acid	4	11	-8	23	13
Inosine	558	390	384	923	347
Uridine	532	221	455	880	559
Thymidine	366	332	437	846	549
Phenylethylamine	21	13	15	25	15
Putrescine	12	18	-20	37	23
2-Aminoethanol	45	18	37	235	101
2,3-Butanediol	17	14	0	35	19
Glycerol	270	125	220	403	170
D,L-α-Glycerol Phosphate	30	23	10	51	34
α-D-Glucose-1-Phosphate	32	1	19	163	36
D-Glucose-6-Phosphate	111	24	49	254	151

APPENDIX H

BIOLOG RAW DATA FOR BEACH *E. COLI* ISOLATES (6-10) AT 24 HR INCUBATION POINT.

	Isolate Type				
Carbon Source	**B(6)**	**B(7)**	**B(8)**	**B(9)**	**B(10)**
Water	0	0	0	0	0
α-Cyclodextrin	24	6	7	12	25
Dextrin	115	183	71	114	201
Glycogen	25	15	19	25	26
Tween 40	35	13	13	-1	22
Tween 80	30	18	22	13	13
N-Acetyl-D-Galactosamine	102	238	145	286	177
N-Acetyl-D-Glucosamine	447	544	476	611	367
Adonitol	9	15	8	27	15
L-Arabinose	699	764	773	556	782
D-Arabitol	26	8	8	39	15
D-Cellobiose	27	-3	6	60	3
i-Erythritol	13	11	2	-8	-5
D-Fructose	415	329	352	517	385
L-Fucose	177	317	155	279	303
D-Galactose	85	145	37	129	254
Gentiobiose	20	29	6	48	25
α-D-Glucose	533	300	308	521	271
m-inositol	27	18	12	35	27
α-D-Lactose	288	283	217	303	292
Lactulose	122	89	130	68	85
Maltose	302	211	201	322	305
D-Mannitol	379	412	427	443	416
D-Mannose	523	423	503	561	355
D-Meliblose	247	286	189	394	172
β-Methyl-D-Glucoside	76	181	119	55	122
D-Psicose	103	110	117	172	115
D-Raffinose	47	63	86	177	36
L-Rhamnose	120	278	252	351	301
D-Sorbitol	149	379	158	136	202
Sucrose	28	30	20	61	13
D-Trehalose	308	414	337	411	440
Turanose	34	27	19	58	22
Xylitol	20	3	12	0	10
Pyruvic Acid Methyl Ester	110	201	92	223	169

Succinic Acid Mono-Methyl Ester	31	21	26	52	27
Acetic Acid	30	51	21	57	15
Cis-Aconitic Acid	22	7	17	8	5
Citric Acid	16	15	16	14	2
Formic Acid	18	2	12	9	12
D-Glatonic Acid Lactone	85	140	81	269	141
D-Glacturonic Acid	125	313	195	467	335
D-Gluconic Acid	320	435	453	534	526
D-Glucosaminic Acid	27	11	11	32	22
D-Glucuronic Acid	210	330	308	557	252
α-Hydroxybutyric Acid	41	20	41	-2	46
β-Hydroxybutyric Acid	38	13	12	14	16
γ-Hydroxybutyric Acid	24	5	1	6	7
ρ-Hydroxy Phenylacetic Acid	84	56	23	1	42
Itaconic Acid	16	7	3	4	11
α-Ketobutyric Acid	40	-14	24	54	20
α-Ketoglutaric Acid	24	14	1	24	18
α-Ketovaleric Acid	38	26	22	21	19
D,L-Lactic Acid	96	172	159	127	167
Malonic Acid	27	17	18	9	19
Propionic Acid	24	86	46	55	29
Quinic Acid	25	1	5	12	8
D-Saccharic Acid	44	100	13	16	19
Sebacic Acid	29	5	19	1	10
Succinic Acid	28	81	76	80	22
Bromosuccinic Acid	10	24	28	48	7
Succinamic Acid	25	20	19	28	12
Glucuronamide	109	146	128	148	131
L-Alaninamide	30	26	13	50	20
D-Alanine	18	30	21	30	22
L-Alanine	24	81	54	68	71
L-Alanyl-glycine	41	69	49	27	103
L-Asparagine	32	131	89	153	91
L-Aspartic Acid	31	111	88	133	56
L-Glutamic Acid	23	19	28	28	26
Glycyl-L-Aspartic Acid	75	48	34	41	112
Glycyl-L-Glutamic Acid	16	23	13	21	11
L-Histidine	9	7	8	12	-9

Hydroxy-L-Proline	12	12	25	21	3
L-Leucine	26	14	19	19	12
L-Ornithine	17	15	2	17	17
L-Phenylalanine	24	11	15	18	6
L-Proline	16	20	18	21	19
L-Pyroglutamic Acid	22	3	8	16	19
D-Serine	15	19	4	19	18
L-Serine	18	12	23	61	49
L-Threonine	35	65	27	55	49
d,L-Carnitine	8	-45	17	15	18
γ-Aminobutyric Acid	12	13	14	28	-1
Urocanic Acid	25	16	2	-8	4
Inosine	386	421	402	258	321
Uridine	239	395	426	364	398
Thymidine	331	372	400	344	353
Phenylethylamine	27	10	20	13	12
Putrescine	36	13	12	8	8
2-Aminoethanol	9	-1	13	13	7
2,3-Butanediol	25	10	13	8	9
Glycerol	66	163	68	95	101
D,L-α-Glycerol Phosphate	80	117	60	70	41
α-D-Glucose-1-Phosphate	517	522	565	514	450
D-Glucose-6-Phosphate	475	573	537	511	409

APPENDIX I

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 6.0 AND 19°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.008	0.02	0.23	0.319	0.7
B(2)	0.009	0.023	0.17	0.275	0.534
B(3)	0.012	0.023	0.237	0.383	0.759
B(4)	0.016	0.025	0.223	0.345	0.771
B(5)	0.019	0.031	0.229	0.373	0.793
B(6)	0.015	0.028	0.244	0.336	0.765
B(7)	0.016	0.034	0.188	0.274	0.556
B(8)	0.016	0.032	0.248	0.341	0.746
B(9)	0.014	0.026	0.228	0.329	0.747
B(10)	0.016	0.026	0.206	0.313	0.665
B(1)-2	0.01	0.025	0.206	0.263	0.588
B(2)-2	0.012	0.021	0.174	0.267	0.44
B(3)-2	0.012	0.021	0.22	0.347	0.653
B(4)-2	0.014	0.021	0.208	0.312	0.643
B(5)-2	0.017	0.023	0.224	0.362	0.684
B(6)-2	0.012	0.025	0.235	0.289	0.637
B(7)-2	0.015	0.028	0.169	0.238	0.457
B(8)-2	0.014	0.027	0.228	0.291	0.645
B(9)-2	0.013	0.024	0.229	0.289	0.635
B(10)-2	0.01	0.027	0.218	0.286	0.606
I(1)	0.009	0.024	0.223	0.418	0.732
I(2)	0.013	0.022	0.198	0.257	0.554
I(3)	0.01	0.022	0.211	0.271	0.522
I(4)	0.015	0.022	0.199	0.253	0.441
I(5)	0.01	0.019	0.174	0.255	0.448
I(6)	0.009	0.021	0.213	0.259	0.44
I(7)	0.011	0.018	0.197	0.263	0.629
I(8)	0.008	0.017	0.134	0.177	0.276
I(9)	0.008	0.016	0.162	0.214	0.427
I(10)	0.008	0.03	0.198	0.28	0.605
I(1)-2	0.009	0.02	0.244	0.392	0.795
I(2)-2	0.007	0.016	0.211	0.289	0.65
I(3)-2	0.007	0.022	0.237	0.311	0.632
I(4)-2	0.007	0.019	0.233	0.29	0.538
I(5)-2	0.013	0.024	0.189	0.303	0.559
I(6)-2	0.013	0.027	0.229	0.305	0.544
I(7)-2	0.007	0.017	0.199	0.292	0.714
I(8)-2	0.009	0.019	0.185	0.247	0.391
I(9)-2	0.009	0.016	0.167	0.266	0.508
I(10)-2	0.007	0.021	0.207	0.331	0.704

APPENDIX J

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 6.8 AND 19°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.008	0.023	0.256	0.335	0.686
B(2)	0.009	0.025	0.194	0.314	0.537
B(3)	0.008	0.022	0.254	0.402	0.741
B(4)	0.009	0.021	0.247	0.358	0.759
B(5)	0.01	0.022	0.256	0.402	0.764
B(6)	0.01	0.022	0.245	0.357	0.734
B(7)	0.009	0.027	0.214	0.287	0.516
B(8)	0.009	0.028	0.251	0.367	0.724
B(9)	0.013	0.031	0.251	0.383	0.745
B(10)	0.011	0.023	0.22	0.338	0.652
B(1)-2	0.01	0.025	0.215	0.282	0.566
B(2)-2	0.014	0.026	0.187	0.277	0.458
B(3)-2	0.012	0.025	0.236	0.361	0.641
B(4)-2	0.014	0.023	0.223	0.32	0.646
B(5)-2	0.012	0.023	0.24	0.358	0.659
B(6)-2	0.013	0.024	0.232	0.316	0.645
B(7)-2	0.01	0.025	0.17	0.229	0.44
B(8)-2	0.013	0.031	0.226	0.336	0.624
B(9)-2	0.012	0.028	0.223	0.334	0.633
B(10)-2	0.011	0.032	0.234	0.283	0.567
I(1)	0.01	0.024	0.232	0.38	0.708
I(2)	0.011	0.022	0.211	0.268	0.526
I(3)	0.009	0.027	0.225	0.297	0.517
I(4)	0.008	0.027	0.23	0.273	0.428
I(5)	0.008	0.021	0.201	0.271	0.452
I(6)	0.008	0.026	0.228	0.282	0.44
I(7)	0.009	0.021	0.199	0.266	0.607
I(8)	0.008	0.017	0.189	0.23	0.378
I(9)	0.007	0.018	0.201	0.271	0.415
I(10)	0.008	0.022	0.191	0.291	0.597
I(1)-2	0.007	0.035	0.266	0.431	0.811
I(2)-2	0.006	0.019	0.25	0.315	0.619
I(3)-2	0.007	0.025	0.236	0.335	0.609
I(4)-2	0.006	0.02	0.245	0.317	0.535
I(5)-2	0.007	0.023	0.219	0.315	0.542
I(6)-2	0.007	0.023	0.226	0.315	0.535
I(7)-2	0.007	0.018	0.209	0.296	0.701
I(8)-2	0.009	0.017	0.183	0.285	0.488
I(9)-2	0.006	0.014	0.201	0.266	0.516
I(10)-2	0.011	0.028	0.198	0.331	0.69

APPENDIX K

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 7.0 AND 19°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.013	0.028	0.238	0.341	0.567
B(2)	0.014	0.026	0.186	0.309	0.536
B(3)	0.016	0.026	0.239	0.397	0.716
B(4)	0.017	0.026	0.234	0.358	0.716
B(5)	0.017	0.023	0.236	0.396	0.717
B(6)	0.021	0.038	0.246	0.368	0.706
B(7)	0.019	0.031	0.201	0.282	0.551
B(8)	0.02	0.029	0.238	0.368	0.693
B(9)	0.016	0.029	0.24	0.377	0.742
B(10)	0.011	0.029	0.208	0.312	0.659
B(1)-2	0.009	0.026	0.239	0.331	0.652
B(2)-2	0.009	0.024	0.191	0.302	0.541
B(3)-2	0.011	0.026	0.252	0.397	0.737
B(4)-2	0.011	0.023	0.243	0.346	0.724
B(5)-2	0.013	0.026	0.254	0.385	0.742
B(6)-2	0.013	0.027	0.255	0.347	0.705
B(7)-2	0.013	0.031	0.196	0.275	0.581
B(8)-2	0.015	0.034	0.252	0.366	0.731
B(9)-2	0.013	0.029	0.25	0.367	0.767
B(10)-2	0.019	0.037	0.198	0.307	0.683
I(1)	0.009	0.025	0.258	0.418	0.822
I(2)	0.009	0.023	0.222	0.314	0.6
I(3)	0.009	0.026	0.235	0.333	0.578
I(4)	0.011	0.028	0.246	0.324	0.53
I(5)	0.009	0.025	0.225	0.319	0.502
I(6)	0.011	0.03	0.245	0.331	0.536
I(7)	0.01	0.024	0.228	0.29	0.681
I(8)	0.01	0.025	0.175	0.258	0.453
I(9)	0.008	0.023	0.212	0.296	0.565
I(10)	0.008	0.03	0.203	0.318	0.695
I(1)-2	0.01	0.027	0.263	0.406	0.791
I(2)-2	0.009	0.022	0.229	0.319	0.59
I(3)-2	0.011	0.024	0.235	0.341	0.566
I(4)-2	0.013	0.027	0.15	0.34	0.503
I(5)-2	0.011	0.024	0.221	0.321	0.494
I(6)-2	0.014	0.028	0.255	0.349	0.529
I(7)-2	0.014	0.026	0.237	0.312	0.676
I(8)-2	0.013	0.022	0.181	0.243	0.404
I(9)-2	0.008	0.02	0.203	0.3	0.485
I(10)-2	0.008	0.023	0.223	0.346	0.654

APPENDIX L

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 8.0 AND 19°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.015	0.032	0.229	0.32	0.597
B(2)	0.012	0.026	0.206	0.304	0.509
B(3)	0.013	0.028	0.238	0.382	0.632
B(4)	0.014	0.025	0.233	0.349	0.615
B(5)	0.013	0.026	0.246	0.387	0.648
B(6)	0.014	0.029	0.147	0.352	0.609
B(7)	0.015	0.031	0.199	0.287	0.489
B(8)	0.014	0.03	0.237	0.348	0.611
B(9)	0.011	0.03	0.22	0.345	0.667
B(10)	0.01	0.027	0.249	0.323	0.606
B(1)-2	0.012	0.027	0.261	0.372	0.697
B(2)-2	0.014	0.029	0.25	0.352	0.592
B(3)-2	0.017	0.03	0.259	0.416	0.716
B(4)-2	0.02	0.031	0.255	0.374	0.725
B(5)-2	0.021	0.032	0.268	0.401	0.734
B(6)-2	0.023	0.035	0.276	0.387	0.745
B(7)-2	0.021	0.036	0.237	0.342	0.549
B(8)-2	0.023	0.039	0.263	0.371	0.727
B(9)-2	0.013	0.03	0.256	0.368	0.726
B(10)-2	0.011	0.035	0.235	0.312	0.614
I(1)	0.012	0.027	0.246	0.394	0.776
I(2)	0.014	0.025	0.219	0.318	0.556
I(3)	0.013	0.028	0.241	0.332	0.564
I(4)	0.014	0.028	0.238	0.329	0.515
I(5)	0.012	0.027	0.228	0.314	0.483
I(6)	0.015	0.032	0.234	0.324	0.543
I(7)	0.018	0.031	0.231	0.302	0.655
I(8)	0.014	0.025	0.179	0.263	0.442
I(9)	0.011	0.022	0.228	0.32	0.559
I(10)	0.009	0.031	0.212	0.33	0.599
I(1)-2	0.011	0.03	0.156	0.384	0.757
I(2)-2	0.011	0.023	0.227	0.313	0.505
I(3)-2	0.011	0.027	0.241	0.342	0.546
I(4)-2	0.011	0.026	0.235	0.323	0.479
I(5)-2	0.011	0.025	0.225	0.314	0.494
I(6)-2	0.012	0.027	0.24	0.325	0.492
I(7)-2	0.01	0.025	0.23	0.306	0.62
I(8)-2	0.011	0.023	0.181	0.258	0.434
I(9)-2	0.01	0.02	0.219	0.293	0.507
I(10)-2	0.01	0.027	0.217	0.351	0.559

APPENDIX M

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 9.0 AND 19°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.015	0.024	0.211	0.268	0.504
B(2)	0.016	0.022	0.203	0.286	0.468
B(3)	0.019	0.026	0.227	0.296	0.507
B(4)	0.014	0.022	0.221	0.297	0.512
B(5)	0.017	0.023	0.228	0.299	0.527
B(6)	0.017	0.027	0.237	0.288	0.504
B(7)	0.016	0.026	0.183	0.248	0.449
B(8)	0.014	0.023	0.216	0.281	0.487
B(9)	0.019	0.029	0.245	0.321	0.609
B(10)	0.015	0.028	0.262	0.308	0.52
B(1)-2	0.014	0.024	0.2	0.28	0.504
B(2)-2	0.016	0.024	0.18	0.286	0.463
B(3)-2	0.025	0.032	0.229	0.319	0.579
B(4)-2	0.019	0.026	0.205	0.267	0.499
B(5)-2	0.017	0.024	0.218	0.269	0.511
B(6)-2	0.019	0.027	0.228	0.27	0.5
B(7)-2	0.017	0.029	0.184	0.239	0.44
B(8)-2	0.018	0.028	0.206	0.258	0.466
B(9)-2	0.014	0.025	0.242	0.283	0.607
B(10)-2	0.014	0.027	0.217	0.265	0.502
I(1)	0.015	0.024	0.202	0.294	0.586
I(2)	0.014	0.023	0.191	0.249	0.362
I(3)	0.014	0.025	0.216	0.271	0.426
I(4)	0.022	0.03	0.249	0.303	0.419
I(5)	0.014	0.023	0.186	0.245	0.37
I(6)	0.014	0.025	0.135	0.23	0.395
I(7)	0.013	0.023	0.22	0.254	0.492
I(8)	0.012	0.021	0.183	0.236	0.38
I(9)	0.011	0.02	0.193	0.237	0.415
I(10)	0.014	0.029	0.154	0.255	0.399
I(1)-2	0.015	0.024	0.197	0.311	0.588
I(2)-2	0.015	0.022	0.203	0.268	0.383
I(3)-2	0.016	0.024	0.219	0.288	0.427
I(4)-2	0.015	0.025	0.206	0.275	0.381
I(5)-2	0.015	0.024	0.189	0.27	0.357
I(6)-2	0.015	0.025	0.132	0.234	0.379
I(7)-2	0.014	0.021	0.217	0.266	0.489
I(8)-2	0.014	0.024	0.187	0.247	0.385
I(9)-2	0.014	0.022	0.204	0.239	0.402
I(10)-2	0.014	0.028	0.176	0.246	0.378

APPENDIX N

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 6.0 AND 37°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.011	0.035	0.223	0.321	0.562
B(2)	0.019	0.062	0.167	0.236	0.46
B(3)	0.011	0.039	0.241	0.451	0.627
B(4)	0.014	0.045	0.232	0.411	0.628
B(5)	0.009	0.044	0.23	0.425	0.665
B(6)	0.011	0.058	0.25	0.382	0.688
B(7)	0.009	0.071	0.174	0.271	0.478
B(8)	0.017	0.083	0.259	0.389	0.652
B(9)	0.009	0.079	0.243	0.385	0.665
B(10)	0.012	0.09	0.286	0.373	0.607
B(1)-2	0.008	0.03	0.227	0.33	0.544
B(2)-2	0.009	0.029	0.154	0.244	0.448
B(3)-2	0.01	0.029	0.261	0.463	0.624
B(4)-2	0.01	0.034	0.243	0.411	0.632
B(5)-2	0.008	0.038	0.26	0.431	0.67
B(6)-2	0.01	0.053	0.263	0.376	0.679
B(7)-2	0.009	0.063	0.18	0.26	0.437
B(8)-2	0.011	0.065	0.262	0.379	0.649
B(9)-2	0.011	0.075	0.27	0.382	0.643
B(10)-2	0.011	0.082	0.282	0.35	0.575
I(1)	0.008	0.032	0.233	0.4	0.67
I(2)	0.008	0.02	0.208	0.312	0.486
I(3)	0.008	0.034	0.23	0.332	0.571
I(4)	0.008	0.035	0.226	0.301	0.433
I(5)	0.008	0.044	0.18	0.268	0.39
I(6)	0.009	0.055	0.234	0.313	0.497
I(7)	0.009	0.062	0.226	0.317	0.635
I(8)	0.007	0.053	0.174	0.22	0.408
I(9)	0.012	0.069	0.195	0.279	0.471
I(10)	0.01	0.08	0.205	0.318	0.54
I(1)-2	0.006	0.026	0.211	0.393	0.663
I(2)-2	0.006	0.026	0.189	0.299	0.479
I(3)-2	0.007	0.042	0.215	0.336	0.577
I(4)-2	0.008	0.045	0.208	0.287	0.423
I(5)-2	0.006	0.051	0.158	0.268	0.377
I(6)-2	0.006	0.057	0.218	0.317	0.484
I(7)-2	0.005	0.06	0.219	0.328	0.626
I(8)-2	0.008	0.066	0.189	0.248	0.394
I(9)-2	0.006	0.072	0.193	0.301	0.448
I(10)-2	0.005	0.087	0.233	0.36	0.536

APPENDIX O

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 6.8 AND 37°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.009	0.04	0.216	0.313	0.513
B(2)	0.01	0.059	0.191	0.272	0.439
B(3)	0.011	0.061	0.26	0.454	0.556
B(4)	0.016	0.068	0.252	0.405	0.588
B(5)	0.011	0.068	0.271	0.445	0.64
B(6)	0.015	0.084	0.282	0.405	0.643
B(7)	0.01	0.101	0.237	0.302	0.429
B(8)	0.01	0.1	0.29	0.423	0.647
B(9)	0.006	0.091	0.278	0.424	0.448
B(10)	0.005	0.103	0.287	0.379	0.536
B(1)-2	0.008	0.04	0.228	0.33	0.525
B(2)-2	0.009	0.055	0.205	0.265	0.444
B(3)-2	0.01	0.051	0.271	0.462	0.561
B(4)-2	0.009	0.059	0.267	0.419	0.585
B(5)-2	0.01	0.068	0.286	0.443	0.636
B(6)-2	0.01	0.082	0.292	0.396	0.639
B(7)-2	0.01	0.096	0.237	0.301	0.427
B(8)-2	0.011	0.103	0.287	0.405	0.651
B(9)-2	0.012	0.09	0.272	0.383	0.675
B(10)-2	0.013	0.95	0.262	0.36	0.576
I(1)	0.011	0.053	0.246	0.384	0.653
I(2)	0.008	0.043	0.219	0.308	0.464
I(3)	0.009	0.048	0.233	0.356	0.572
I(4)	0.012	0.052	0.238	0.326	0.423
I(5)	0.009	0.045	0.199	0.289	0.421
I(6)	0.008	0.044	0.237	0.303	0.479
I(7)	0.012	0.059	0.23	0.326	0.566
I(8)	0.013	0.048	0.192	0.226	0.413
I(9)	0.009	0.056	0.192	0.281	0.45
I(10)	0.008	0.049	0.192	0.307	0.472
I(1)-2	0.009	0.043	0.239	0.413	0.647
I(2)-2	0.009	0.047	0.223	0.313	0.457
I(3)-2	0.008	0.058	0.232	0.376	0.561
I(4)-2	0.007	0.061	0.232	0.335	0.414
I(5)-2	0.007	0.07	0.2	0.31	0.402
I(6)-2	0.007	0.073	0.243	0.34	0.475
I(7)-2	0.007	0.078	0.222	0.348	0.573
I(8)-2	0.009	0.078	0.192	0.274	0.434
I(9)-2	0.007	0.096	0.215	0.339	0.458
I(10)-2	0.008	0.105	0.231	0.365	0.512

APPENDIX P

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 7.0 AND 37°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.01	0.039	0.222	0.334	0.52
B(2)	0.009	0.035	0.184	0.263	0.446
B(3)	0.01	0.032	0.27	0.425	0.543
B(4)	0.011	0.043	0.267	0.389	0.567
B(5)	0.011	0.06	0.279	0.414	0.601
B(6)	0.013	0.061	0.281	0.375	0.601
B(7)	0.011	0.068	0.215	0.288	0.45
B(8)	0.013	0.075	0.28	0.388	0.606
B(9)	0.014	0.088	0.282	0.385	0.644
B(10)	0.014	0.096	0.275	0.364	0.547
B(1)-2	0.011	0.035	0.207	0.326	0.482
B(2)-2	0.012	0.032	0.208	0.302	0.456
B(3)-2	0.013	0.035	0.25	0.428	0.537
B(4)-2	0.013	0.036	0.256	0.38	0.526
B(5)-2	0.013	0.048	0.258	0.418	0.596
B(6)-2	0.014	0.057	0.262	0.378	0.586
B(7)-2	0.014	0.058	0.215	0.284	0.434
B(8)-2	0.014	0.071	0.261	0.392	0.593
B(9)-2	0.014	0.073	0.251	0.376	0.623
B(10)-2	0.014	0.087	0.248	0.386	0.525
I(1)	0.011	0.039	0.252	0.405	0.597
I(2)	0.012	0.036	0.219	0.311	0.414
I(3)	0.011	0.039	0.234	0.375	0.501
I(4)	0.012	0.047	0.232	0.345	0.367
I(5)	0.011	0.057	0.23	0.32	0.355
I(6)	0.011	0.064	0.258	0.351	0.445
I(7)	0.011	0.077	0.244	0.342	0.493
I(8)	0.011	0.056	0.202	0.263	0.344
I(9)	0.013	0.077	0.227	0.335	0.417
I(10)	0.013	0.077	0.216	0.345	0.479
I(1)-2	0.009	0.043	0.258	0.38	0.629
I(2)-2	0.009	0.032	0.224	0.307	0.426
I(3)-2	0.008	0.041	0.24	0.343	0.514
I(4)-2	0.009	0.049	0.249	0.338	0.392
I(5)-2	0.009	0.081	0.221	0.31	0.443
I(6)-2	0.01	0.07	0.276	0.345	0.462
I(7)-2	0.011	0.07	0.259	0.342	0.515
I(8)-2	0.009	0.077	0.213	0.267	0.417
I(9)-2	0.013	0.097	0.269	0.349	0.449
I(10)-2	0.013	0.098	0.247	0.346	0.503

APPENDIX Q

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 8.0 AND 37°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.013	0.036	0.219	0.325	0.465
B(2)	0.011	0.038	0.199	0.286	0.43
B(3)	0.012	0.046	0.259	0.366	0.455
B(4)	0.012	0.052	0.261	0.358	0.489
B(5)	0.012	0.064	0.281	0.382	0.517
B(6)	0.013	0.075	0.295	0.379	0.535
B(7)	0.014	0.09	0.233	0.315	0.436
B(8)	0.014	0.097	0.288	0.389	0.515
B(9)	0.017	0.109	0.273	0.384	0.577
B(10)	0.016	0.115	0.288	0.342	0.488
B(1)-2	0.012	0.035	0.213	0.303	0.403
B(2)-2	0.014	0.037	0.215	0.292	0.426
B(3)-2	0.015	0.043	0.257	0.371	0.44
B(4)-2	0.015	0.051	0.259	0.355	0.472
B(5)-2	0.013	0.054	0.262	0.371	0.484
B(6)-2	0.014	0.064	0.266	0.37	0.505
B(7)-2	0.012	0.065	0.228	0.295	0.406
B(8)-2	0.015	0.068	0.251	0.372	0.473
B(9)-2	0.015	0.075	0.247	0.382	0.556
B(10)-2	0.016	0.079	0.245	0.345	0.436
I(1)	0.013	0.035	0.21	0.338	0.475
I(2)	0.013	0.033	0.192	0.301	0.362
I(3)	0.014	0.049	0.233	0.355	0.427
I(4)	0.014	0.046	0.211	0.32	0.336
I(5)	0.012	0.041	0.219	0.325	0.421
I(6)	0.013	0.034	0.217	0.306	0.396
I(7)	0.011	0.044	0.206	0.306	0.4
I(8)	0.014	0.041	0.2	0.274	0.361
I(9)	0.016	0.041	0.203	0.297	0.362
I(10)	0.013	0.044	0.158	0.308	0.392
I(1)-2	0.011	0.045	0.231	0.336	0.518
I(2)-2	0.011	0.047	0.217	0.303	0.394
I(3)-2	0.015	0.055	0.26	0.353	0.451
I(4)-2	0.012	0.053	0.25	0.335	0.35
I(5)-2	0.011	0.055	0.23	0.316	0.464
I(6)-2	0.013	0.052	0.256	0.319	0.409
I(7)-2	0.012	0.062	0.249	0.3	0.42
I(8)-2	0.013	0.06	0.224	0.248	0.37
I(9)-2	0.013	0.073	0.25	0.324	0.404
I(10)-2	0.014	0.073	0.228	0.309	0.414

APPENDIX R

OPTICAL DENSITY DATA FOR BEACH AND INTESTINAL *E. COLI* ISOLATES AT PH 9.0 AND 37°C. (**-2 INDICATES ISOLATE REPLICATE)

	Incubation Time Point (hr)				
Isolate	T=0	T=2	T=6	T=10	T=24
B(1)	0.013	0.015	0.144	0.2	0.329
B(2)	0.013	0.011	0.12	0.193	0.359
B(3)	0.014	0.019	0.181	0.231	0.352
B(4)	0.013	0.023	0.173	0.23	0.364
B(5)	0.015	0.029	0.175	0.23	0.373
B(6)	0.014	0.04	0.202	0.244	0.379
B(7)	0.015	0.053	0.195	0.222	0.31
B(8)	0.016	0.058	0.158	0.24	0.327
B(9)	0.016	0.07	0.198	0.252	0.389
B(10)	0.016	0.076	0.201	0.262	0.312
B(1)-2	0.012	0.017	0.151	0.207	0.353
B(2)-2	0.014	0.019	0.132	0.216	0.341
B(3)-2	0.014	0.027	0.183	0.243	0.331
B(4)-2	0.014	0.038	0.207	0.257	0.368
B(5)-2	0.014	0.041	0.214	0.249	0.359
B(6)-2	0.016	0.058	0.233	0.271	0.378
B(7)-2	0.017	0.063	0.18	0.229	0.294
B(8)-2	0.017	0.074	0.202	0.262	0.316
B(9)-2	0.016	0.087	0.157	0.297	0.399
B(10)-2	0.015	0.086	0.152	0.279	0.351
I(1)	0.013	0.023	0.138	0.206	0.367
I(2)	0.013	0.025	0.133	0.195	0.279
I(3)	0.014	0.039	0.186	0.251	0.356
I(4)	0.013	0.035	0.195	0.234	0.291
I(5)	0.012	0.032	0.172	0.212	0.31
I(6)	0.013	0.035	0.092	0.169	0.316
I(7)	0.016	0.051	0.236	0.25	0.373
I(8)	0.014	0.039	0.159	0.192	0.319
I(9)	0.012	0.035	0.156	0.194	0.268
I(10)	0.013	0.036	0.162	0.205	0.25
I(1)-2	0.011	0.017	0.121	0.192	0.33
I(2)-2	0.011	0.015	0.124	0.184	0.239
I(3)-2	0.011	0.027	0.176	0.234	0.304
I(4)-2	0.011	0.028	0.179	0.228	0.241
I(5)-2	0.012	0.041	0.149	0.21	0.248
I(6)-2	0.011	0.048	0.062	0.177	0.272
I(7)-2	0.014	0.063	0.195	0.262	0.319
I(8)-2	0.012	0.06	0.114	0.215	0.258
I(9)-2	0.012	0.069	0.157	0.241	0.257
I(10)-2	0.011	0.077	0.152	0.242	0.264

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