FITNESS OF *ESCHERICHIA COLI* WHEN IN MIXED CULTURE WITH *ENTEROCOCCUS FAECALIS*

by

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DEDICATION

This thesis is dedicated to my late grandfather, Erwin Sultemeier, for his continuous support and pride in my accomplishments.

ACKNOWLEDGEMENTS

I would like to Thank Dr. McLean for his mentorship and support throughout this journey. I would also like to thank my fellow lab mates Candace Longoria, Starla Thornhill, Sara Robertson, Keith Garcia, and Kerri White for reminding me to stay positive and keep working hard. I would like to thank my committee members Dr. Kakirde and Dr. Palmer of UT Dallas. I would also like to thank my fiancé, Dakota Howell, for his continuous support and love. Lastly, I would like to thank my family for always standing behind me and their relentless love.

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LIST OF ABBREVIATIONS

Abbreviation	Description
Agg	Aggregation substance
AHL	*N*-acyl-homoserine lactone
EfbA	Enterococcal fibronectin-binding protein
Esp	Enterococcal surface protein
gelE	Gelatinase
LB	Luria bertani
LUTS	Lower urinary tract symptoms
MCM	methylmalonoyl CoA mutase
ORF	Open reading frame
PAI	Pathogenicity island
Sbm	Sleeping beauty mutase
UTI	Urinary tract infection
VRE	Vancomycin resistant enterococci

ABSTRACT

Escherichia coli (*E. coli*) coexists with many different species, such as *Enterococcus faecalis (E. faecalis)*, in the gastrointestinal tract of many animals. Under normal circumstances, the two bacteria live alongside each other and a multitude of other microorganisms without causing infection. However, there are occasional instances when an imbalance occurs and certain flora are able to outcompete the rest. These superior bacteria express specific traits that allow them to increase colonization and infect the host organism. There is currently little known about the mechanism of how *E. coli* is able coexist with *E. faecalis*. Using the Keio collection of *E. coli*, we identified that the gene yliK in *E. coli,* commonly known as methylmalonoyl CoA mutase, that is essential for its growth when in mixed culture with *E. faecalis*. Methylmalonoyl CoA mutase is part of a four gene operon encoding for enzymes that convert succinate into propionate. The *E. coli* mutant pure culture exhibited increased fitness with the addition of propionate. The *E. faecalis*, although at a much lower cell density, also exhibited increased fitness with the addition of propionate. The *E. coli* mutant *sbm*/*E. faecalis* mixed culture showed to have increased fitness when grown together as when compared to the fitness of the two bacteria in pure culture. The *E. coli* mutant *sbm*/*E. faecalis* mixed culture also showed an increase in fitness with the addition of propionate. These results suggest that *E. coli* and *E. faecalis* increase the other's fitness, and that this elevated fitness is enhanced when propionate is added to the environment.

I. INTRODUCTION

Escherichia coli (*E. coli*) is a rod-shaped, gram negative, facultative anaerobe, commonly found in the environment, food, and gastrointestinal tract of many species. The bacterium belongs to the genus *Escherichia*. Classification of pathogenic *E. coli* is generally based on the presence or absence of pathogenicity islands (PAI's) that have been obtained through horizontal gene transfer (Landraud et al. 2013). These regions code for a variety of virulence factors such as capsules, toxins, and adhesins (Lloyd et al. 2007). Based off of phylogenetic analysis, *E. coli* can be divided into four different groups: A, B1, B2, and D. B2 is the most virulent class followed by D, and then A and B1 (Landraud et al. 2013). Certain strains of *E. coli* are capable of producing cytotoxins that can cause mild to severe symptoms in humans. These strains are food borne pathogens and can be transmitted to humans from animal reservoirs via ingestion of contaminated water or foods (Miko et al. 2009).

Uropathogenic *E. coli* is able to produce two types of pili. The type 1 pilus attaches to mannosylated uroplakin proteins on the luminal side of the bladder (Zhour et al. 2007). P pili bind to globoseries gylcolipids on the epithelial surface of the kidneys (Dodson et al. 1993). *E. coli* has shown to evade the immune system by forming a niche inside superficial epithelial cells (Hunstad, Justice 2010). Biofilms formed by *E. coli* allow the bacteria to adhere to surfaces, provide protection from the environment and antimicrobials, and enhance genetic exchanges (Watnick and Kolter 2000). There are five stages of biofilm development (Sauer et al. 2002). The first stage is termed reversible attachment wherein the planktonic growth initially adheres to a surface but is not permanently attached. During the second stage is called irreversible attachment; the

bacteria now form adhesins such as pili to permanently attach to the surface. The third stage is maturation 1; the biofilm becomes encased and produces a "slime". The fourth stage is maturation 2; secretion increases and the formation of water channels within the biofilm occurs. The last stage is the release stage; this is when the biofilm releases new planktonic growth to adhere to other surfaces.

Enterococcus faecalis is a spherical, non-motile, gram positive normal flora of the gastrointestinal tract of humans and animals, but also can act as an opportunistic pathogen. It is of the genus *Enterococcus*, previously known as Group D *Streptococcus*. It is the leading species to causes disease in humans in the *Enterococcus* genus (McBride et. al 2007). The *enterococcus* species is responsible for approximately 12% of nosocomial infections acquired in the United States (Torelli et. al 2012).

E. faecalis has several characteristics that contribute to its virulence. Gelatinase (*gelE*), aggregation substance (*Agg*), enterococcal surface protein (*Esp*), cytolysin toxin, and a hyaluronidase are all traits that have been proven or are suspected to enhance *E. faecalis*' virulence. It has been shown that *E. faecalis* is capable of acquiring new genetic traits through horizontal gene transfer. Some of these new traits include different virulence and antibiotic resistance genes which help the bacteria survive in different and complex environments (Mundy et. al 2000). Gelatinase is an extracellular zinc metallo-endopeptidase that can hydrolyse gelatin, casein, hemoglobin and other bioactive peptides (Coque et. al 1995). The gene *sprE* codes for serine protease and is cotranscribed with *gelE*. Quorum-sensing system encoded by the *fsr* locus regulates the transcription of these two genes (Sifri et. al 2002). Quorum-sensing occurs when the bacterial population produces a signal via an autoinducing peptide (AIP). Once AIP

reaches a threshold concentration, it interacts with cell surface receptors or reenters the cell and causes a transcriptional regulation cascade.

The surface of *E. faecalis* contains aggregation substance. Agg has shown to promote cell aggregation, possibly contributing to the pathogenicity of the bacteria. In the presence of *Agg*, the hydrophobicity of the cell is enhanced. Cholesterol then localizes to phagosomes and is thought to delay or prevent fusion with lysosomal vesicles. This prevents the pathogen from being digested and killed.

Along with *GelE* and *Agg*, *E. faecalis* has an extracellular surface protein (*Esp*). *Esp* is a cell wall associated protein that promotes adhesion, colonization and evasion of the immune system. It is also thought to play a role in antibiotic resistance. It is found in high numbers in infection derived isolates. Another essential characteristic of *Esp* is biofilm formation. It is responsible for the formation of pili which are necessary for the production of biofilm. Recent studies have shown *E. coli* and *E. faecalis* (Ghosh et al. 2013) growing together in the gastrointestinal tracts of young kittens, proving that the two bacteria are able to coexist. However, the mechanism is not clearly understood.

Urinary tract infections are considered the most common bacterial infection (Foxman, Betsy 2003). Almost one third of women will have sought medical attention for a self-limiting urinary tract infection by the age of 24. Of this group, between 15-25% of these women will suffer from recurrent or a chronic form of UTI. *E. coli* is responsible for up to 90% of diagnosed nosocomial and community acquired bladder infection cases (Horsley et. al 2013). As prevalent as UTI's are, tests that screen for UTI's are very inadequate, especially in patients that do not present classic acute infective symptoms (Horsley et. al 2013). With this is mind, it is quite possible that patients that receive

negative routine test results may in fact have a low-grade bacterial infection. Lower urinary tract symptoms (LUTS) describes a range of urological manifestations, including pain in the lower urinary tract and symptoms of urine storage and voiding. *E. coli* is the most prevalent UTI-causing bacteria, however that is not the case for LUTS. *E. faecalis* is the most cell-associated pathogen in this disease. Urothelial shedding is often seen in UTI and LUTS bladders. Recent studies have shown that *E. coli* formed very tight biofilms on the surface of the shed bladder epithelial cells but nothing inside of the cells (Horsely et. al 2013). However, *E. faecalis* formed looser, more disperse clusters on the surface of the urothelial cells. More organized colonies were present but were closer to the nuclei suggesting cellular invasion (Horsely et. al 2013). Cellular invasion makes *E. faecalis* a more difficult pathogen to treat.

Enterococci are the third most common pathogens isolated from bloodstream infections (Koch et. al 2004). Bacteremia can cause serious complications especially in immunocompromised patients. As *E. faecalis* often acts as an opportunistic pathogen in these patients, bacteremia becomes an even more complicated matter. Surviving in the bloodstream is not something that all pathogens can accomplish. However, the physiology of *E. faecalis* is well adapted to the bloodstream, enabling it to survive the oxidative stress and high pH and salt concentrations in that environment.

As enterococcal infection rates continue to rise, so do the number of high level resistance to numerous antimicrobials. The global infectious diseases community is distressed from the emergence of vancomycin-resistant enterococci (VRE). Few options are left for treatment of enterococcal vancomycin-resistance. The transfer of genes from enterococci to *Staphylococcus aureus* has been confirmed; different selection pressures

for VRE proliferation, and lastly the few successful attempts of prevention and treatment methods for containing the vancomycin resistance all accentuate the difficulty of containing the issue once it is established (Mundy et. al 2000). The biofilm forming virulence trait of *E. coli* and *E. faecalis* make the pathogens up to 1,000 fold more resistant to antimicrobials (Furustrand et. al 2011). Studies have shown some strains of *E. coli* to be 97.5% resistant to ampicillin, 65% resistant to streptomycin, 57% resistant to sulfamethoxazole-trimethobrim and 50% resistant to tetracycline (Saqr et al. 2016). Recently, a strain of *E. coli* has been identified in Pennsylvania that has not responded to the last resort antibiotic colistin.

There are over 1,000 different species (Lee et al. 2015) that are considered normal flora of the gastrointestinal tract. The gastrointestinal tract is an important source of bacteria that affects immunity (Lee et al. 2015) and/or may cause infection. The enterobacterium, *E. coli,* has shown to be the main colonizer of the human gut (Elliot et al. 1999). Under normal conditions, the subpopulations of bacteria are able to coexist and not cause issues for the host. However, there are many factors that affect which bacteria are present and their abundance, such as age, diet, health, and environment. Studies have shown a decrease in diversity of bacteria and a rise in bacteria such as *E. coli* and *E. faecalis* in sick or distressed individuals (Ghosh et al. 2013). This change in equilibrium is what allows certain bacteria to overpopulate and cause complications for the host.

It is important to acknowledge that many infections are caused by more than one agent, and understanding how they work together is essential in figuring out how to treat the infection. Studies have shown an increase in antibiotic resistance of polymicrobial infections when compared to monomicrobial infections (Dalton et al. 2011). *P.*

aeruginosa is known to produce several different bactericidal factors thought to enhance its competitiveness over other bacteria, but a recent study showed *P. aeruginosa* living at steady levels in a mixed biofilm growth with *S. aureus, E. faecalis,* and *Fascioloides magna (F. magna)* (Dalton et al. 2011). Gene transfer may be responsible for equilibrium. Ricardo Torelli, Pascale Serror, et. al observed the first characterization of a *PavA*-like fibronectin-binding protein in *E. faecalis* termed enterococcal fibronectin-binding protein (*EfbA*). *PavA* is a pneumococcal adherence and virulence factor of *Streptococcus pneumoniae*. The protein facilitates pneumococcal binding to immobilized fibronectin. Mutations or deficiencies of *PavA* reduce the ability of the pneumococci to adhere or invade epithelial and endothelial cells in humans, significantly decreasing the bacteria's virulence (Torelli et. al 2012). The 71% similarity *EfbA* has to *S. pneumoniae PavA* suggests the gene was acquired through horizontal gene transfer. It has been shown that a deletion of *EfbA* strongly decreased the ability of the mutant strain to adhere to immobilized human fibronectin. *EfbA* promotes specific adherence of the pathogen to fibronectin. Fibronectin is a molecular bridge for the adherence of several Gram-positive cocci. It is also considered a signal transduction trigger which is then able to lead to bacterial host invasion.

Another possibility that allows normally virulent bacteria to coexist is virulence trait control. This is when one bacteria is able to enhance or decrease another's virulence traits. Studies have shown that when *E. coli* is grown in mixed culture with *P. aeruginosa,* it is able to inhibit quorum sensing and therefore survive alongside the otherwise fatal bacterium. The indole produced by *E. coli* inhibits the pyocyanin production and other *N*-acyl-homoserine lactones (AHL's) regulated virulence factors of

P. aeruginosa (Chu et al. 2012).

Genome sequencing has allowed for a better understanding of organism at a molecular level. Although *E. coli* K-12 is one of the most studied and characterized organisms, there is still much left unknown. The Keio collection is a group of single nonessential gene knockout *E. coli* K-12 BW25113 mutants. The genome contains 4453 genes that encode for 4296 open reading frames (ORF's), 156 RNA's, and one annotated feature. The collection was created by replacing the ORF's with a kanamycin cassette lined with a FLP recognition target site. This was accomplished through a one-step method for inactivation of the chromosomal primers and genes that are designed to create the in-frame deletions upon removal of the kanamycin resistance cassette (Baba et. al 2006). One of the mutants created in this collection no longer codes for methylmalonoyl CoA mutase (MCM). MCM, also known as sleeping beauty mutase (sbm). Methylmalonoyl-CoA mutase is part of a four gene operon, *sbm-ygfD-ydfG-ygfH,* encoding for enzymes that convert succinate into propionate (Froese et. Al 2010).

E. coli and *E. faecalis* are both normal inhabitants of the gastrointestinal tract of both humans and many animals. Under normal circumstances, the two bacteria live alongside each other and a multitude of other microorganisms without causing infection. However, there are occasional instances when an imbalance occurs and certain flora are able to outcompete the rest. These superior bacteria express specific traits that allow them to increase colonization and infect the host organism. *E. coli* and *E. faecalis* are able to coexist during this bacterial imbalance. This study observes the traits that allow this coexistence to happen.

II. MATERIALS AND METHODS

Strains and Culture Conditions

The bacterial strains used were *E. faecalis* OG1RF, *E. coli* BW25113, *E. coli tnaA*, and the *E. coli* Keio collection (Baba et al. 2006). Broth cultures of *E. faecalis*, BW25113, and *tnaA* were grown in Luria Bertani (LB) at 37C for 24 hours. Plate cultures of *E. faecalis* were grown on HiChrome agar, which distinguishes *E. faecalis* in mixed populations, BW25113 and the Keio collection on LB agar, and *tnaA* on LB+Kanamycin agar.

Screening

E. faecalis, BW25113, and *tnaA* were grown in 10 mL of LB at 37C for 24 hours. 100uL of LB was added to each well of a 96 well plate. One plate of *E. coli* mutants was then transferred to the experimental plate using a 96 well replicator. 100uL of *E. faecalis*, BW25113, and tnaA were added to the corresponding experimental 96 well plates and then incubated at 37C for 24 hours. 2uL of inoculated broth was then plated on the corresponding selective media and LB agar and incubated at 37C for 24 hours. Plates were then observed for increase or decrease in fitness.

Growth Curve

Overnight cultures of *E. faecalis*, BW25113, tnaA, and the *E. coli* mutant, methylmalonoyl CoA mutase(sbm) were added at 1% and 10% concentrations to 0% propionate LB, 0.9134mg/L propionate LB, 1.8263mg/L propionate LB, and 3.6526mg/L propionate LB in a microtiter plate in both pure and mixed cultures. Plates were incubated for 48 hours, with OD600 readings taken every 20 minutes.

OD600 Calibration Curves

Pure and mixed cultures of *E. faecalis*, BW25113, tnaA, and *E. coli* mutant *sbm* were grown overnight in 0mg/L propionate LB, 0.9134mg/L propionate LB, 1.8263mg/L propionate LB, and 3.6526mg/L propionate LB. The cultures were then serially diluted into LB and plated on respective agar plates. The plates were incubated at 37C for 24 hours and then observed for CFU's per mL for each OD600 value. Pure and mixed cultures of *E. faecalis*, BW25113, tnaA, and *E. coli* mutant sbm were grown for 48 hours in 0mg/L propionate LB, 0.9134mg/L propionate LB, 1.8263mg/L propionate LB, and 3.5626mg/L propionate LB. The cultures were then serially diluted into LB and plated on respective agar plates. The plates were incubated at 37C for 24 hours and then observed for CFU's per mL for each OD600 value.

Biolog

An isolation streak was performed on two different blood agar plates, each with a different colony from a mixed culture *E. faecalis/E. coli* mutant *sbm* LB plate. The blood agar plates were incubated at 37C for 24 hours. Individual colonies were then isolated and transferred into IF media tubes until the desired turbidity was reached. The two inoculated IF tubes were then transferred into two different MicroPlate, 100uL per well. The plate was then covered and incubated at 30C for 24 hours.

III. RESULTS

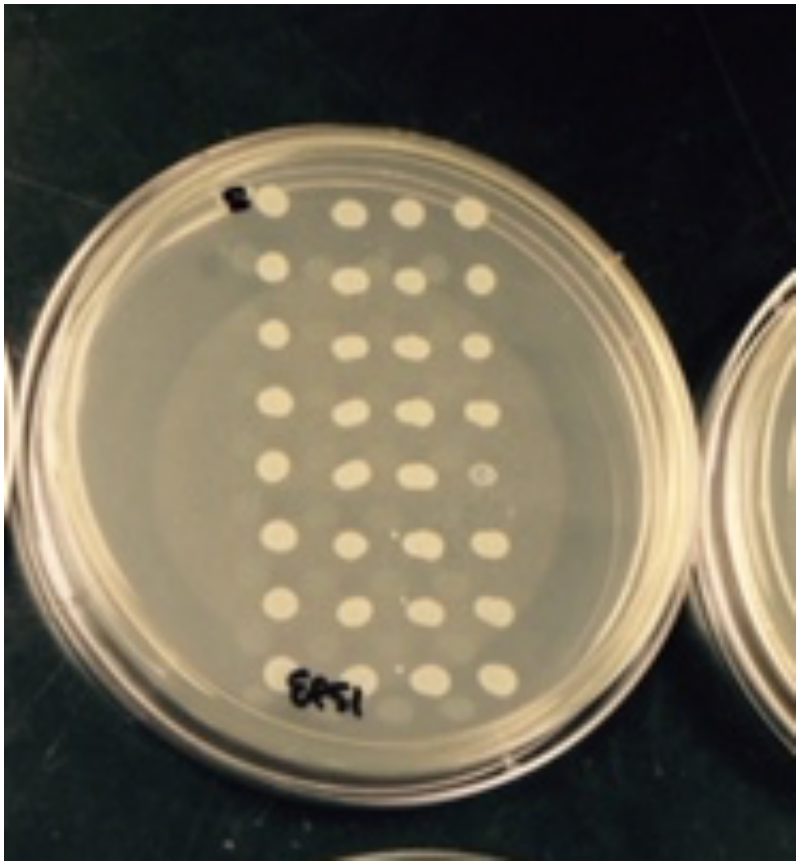

Figure 1. Screening Process. Decreased growth is observed in the mixed culture *E. faecalis*/*E. coli sbm* mutant.

Screening

Through the screening process, methyl-malonoyl CoA mutase appeared to be essential for *E. coli's* survival when in mixed culture with *E. faecalis*. Decreased growth was observed on the LB plate when compared to the same well growth on the HiChrome agar (Figure 1).

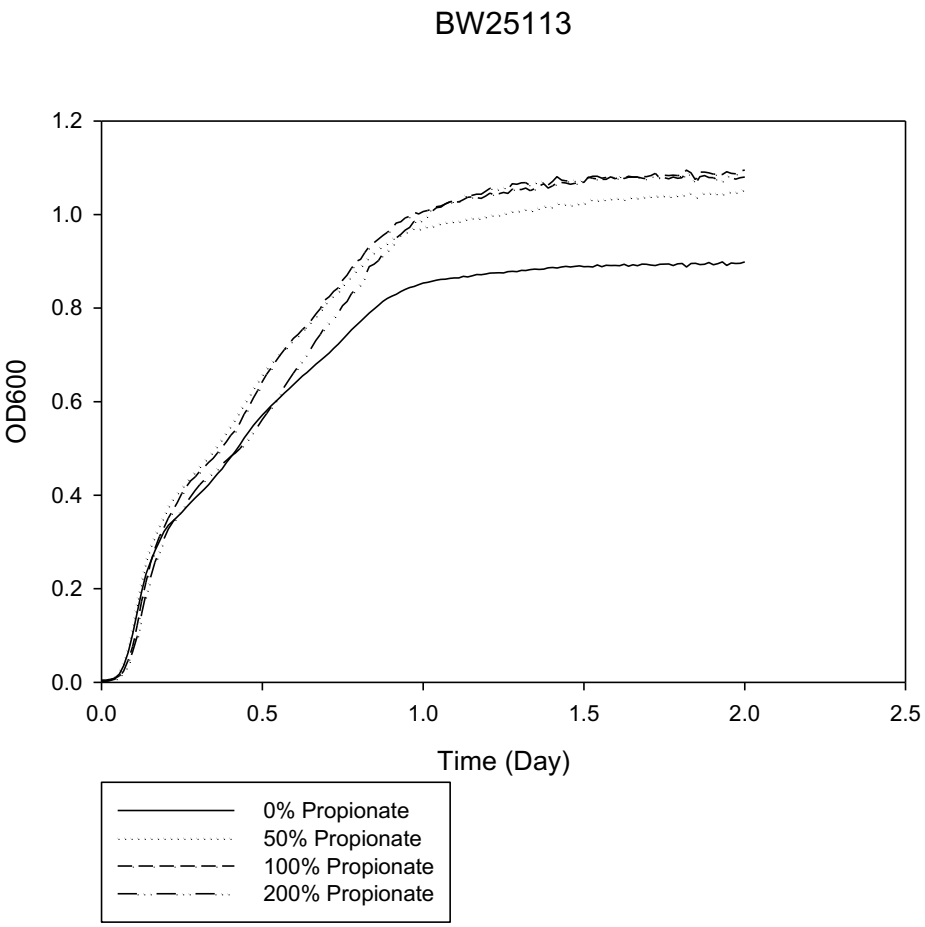

Figure 2. 1% BW25113 with Propionate. *E. coli* BW25113 showed increased fitness with increased propionate levels, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

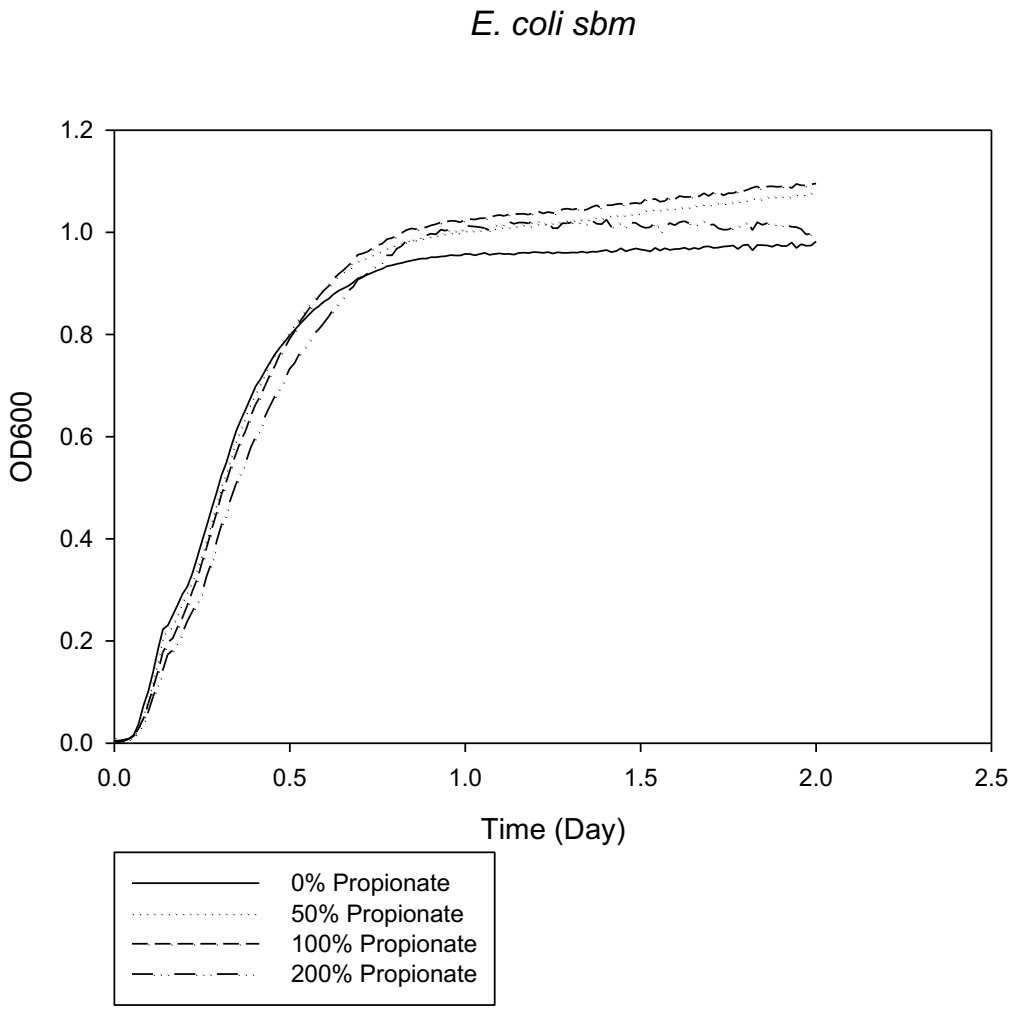

Figure 3. 1% *E. coli sbm* with Propionate. *E. coli sbm* showed increased fitness with increased propionate levels, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

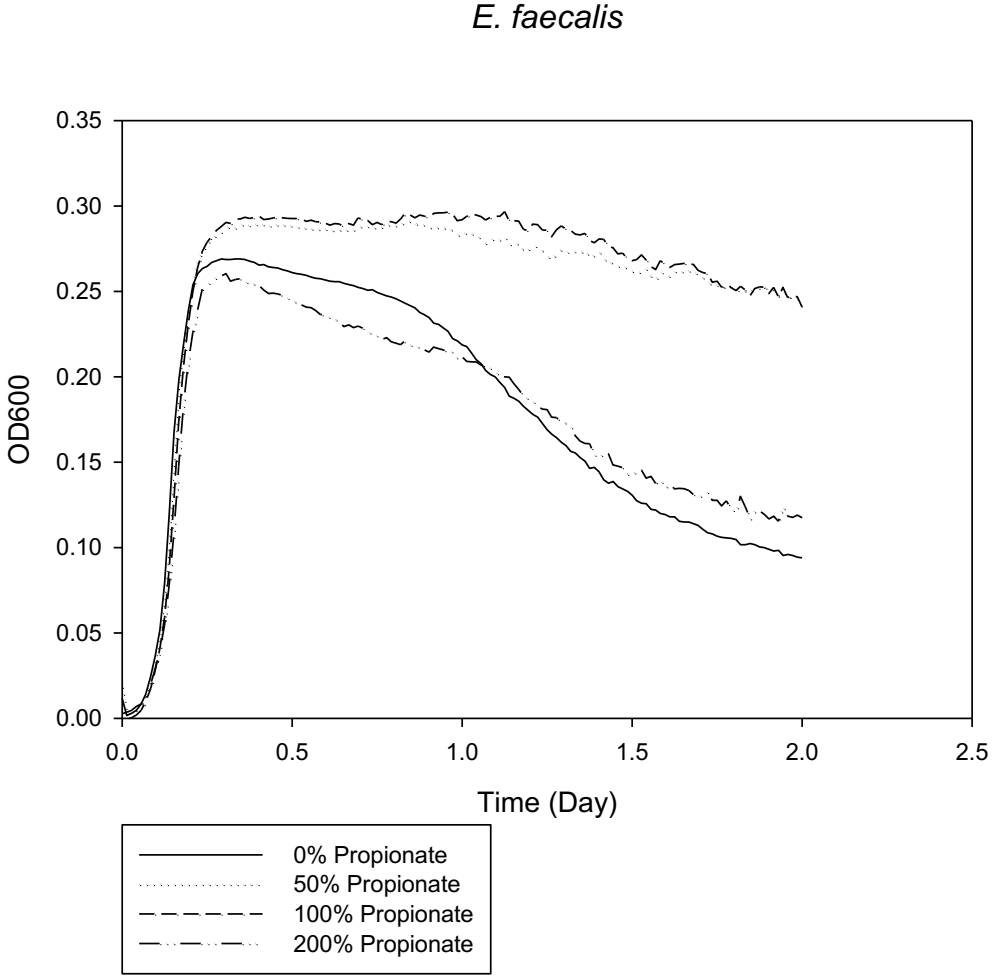

Figure 4. 1% *E. faecalis* with Propionate. *E. faecalis* showed optimal growth with 0.9134mg/L and 1.8263mg/L propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

1% Inoculum

BW25113 showed optimal fitness when grown with 0.9134mg/L, 1.8263mg/L, and 3.5626mg/L of propionate (Figure 2). The *E. coli* mutant also showed increased fitness when grown with the addition of propionate (Figure 3). *E. faecalis* showed optimal levels of fitness when grown with the addition of 0.9134mg/L and 1.8263mg/L propionate (Figure 4). When grown in mixed culture, the BW25113 and *E. coli* mutant grew best with the addition of propionate (Figure 5). The *E. faecalis* and *E. coli* mutant

mixed culture did not show any significance between any of the the levels of propionate (Figure 6). *E. faecalis* grew at a much lower density than the rest of the cultures throughout all levels of propionate addition (Figure 7).

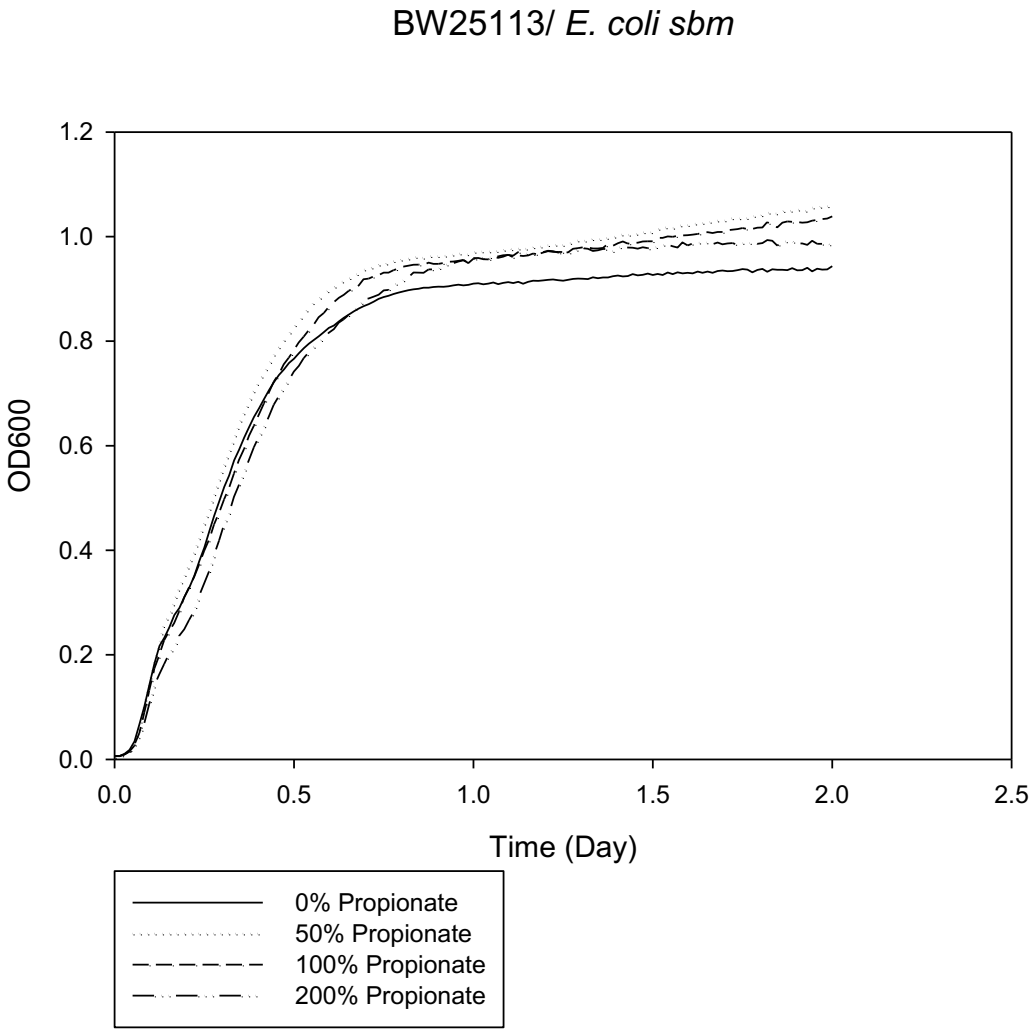

Figure 5. 1% BW25113/ *E. coli sbm* Mixed Culture with Propionate. The mixed culture of *E. coli* BW25113/*E. coli* mutant *sbm* showed increased fitness with the addition of propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

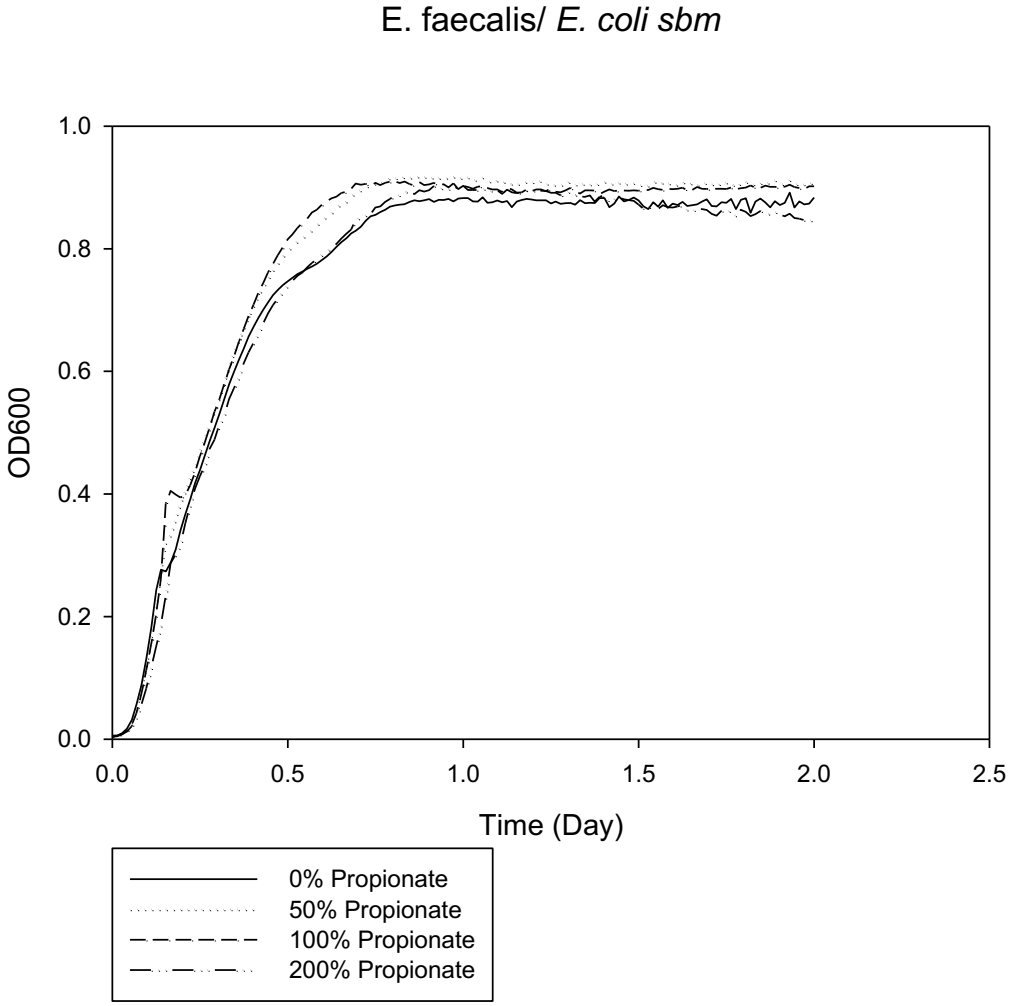

Figure 6. 1% *E. faecalis*/ *E. coli sbm* Mixed Culture with Propionate. The mixed culture of *E. faecalis*/*E. coli* mutant *sbm* showed increased fitness with the addition of propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

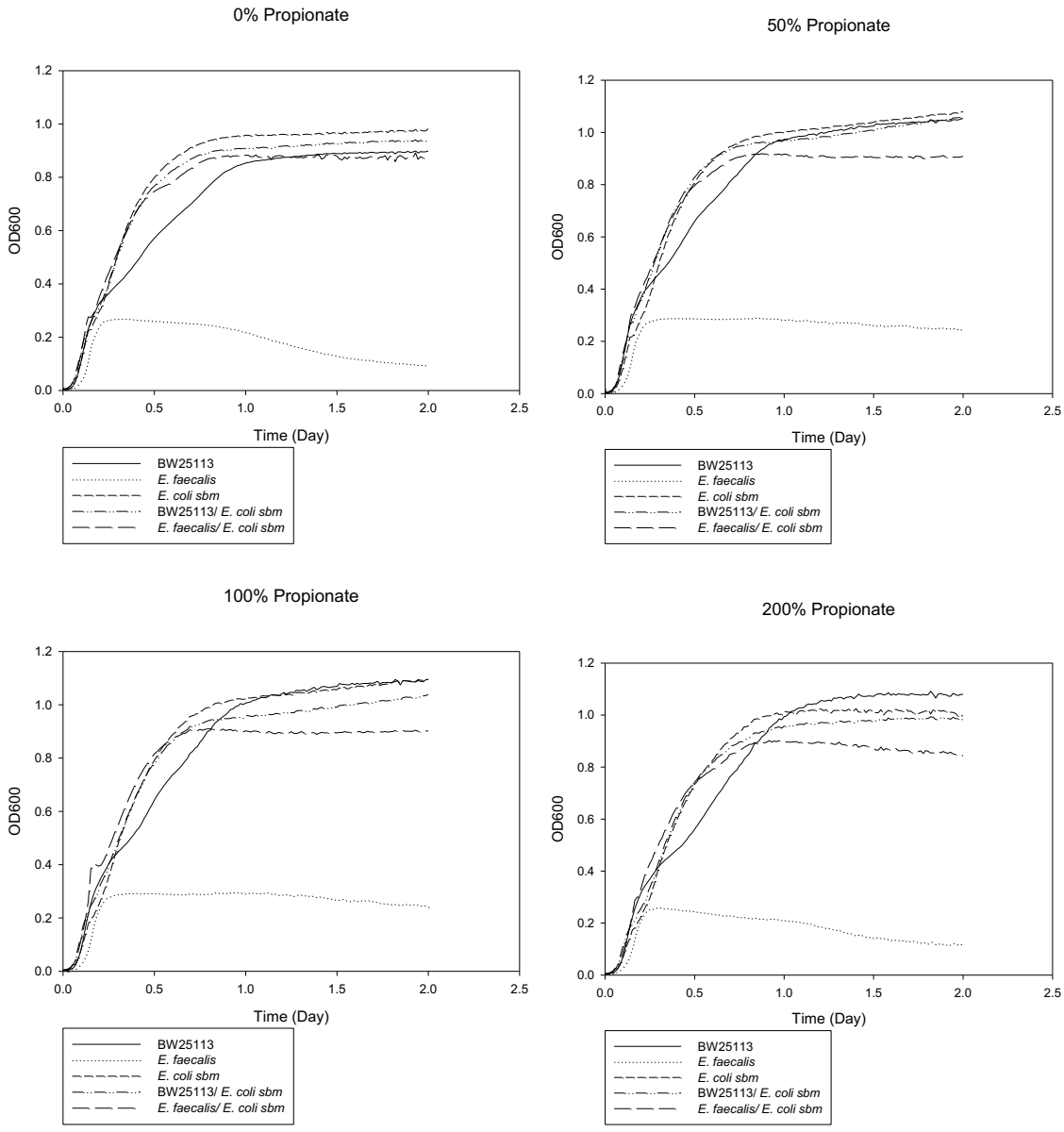

Figure 7. 1% Inoculum Growth within Propionate Levels. *E. faecalis* grew at much lower cell densities than the two *E. coli* strains. As propionate levels increased, growth of both *E. coli* strains increased. *E. faecalis* showed optimal growth at 0.9134mg/L and 1.8263mg/L propionate levels, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

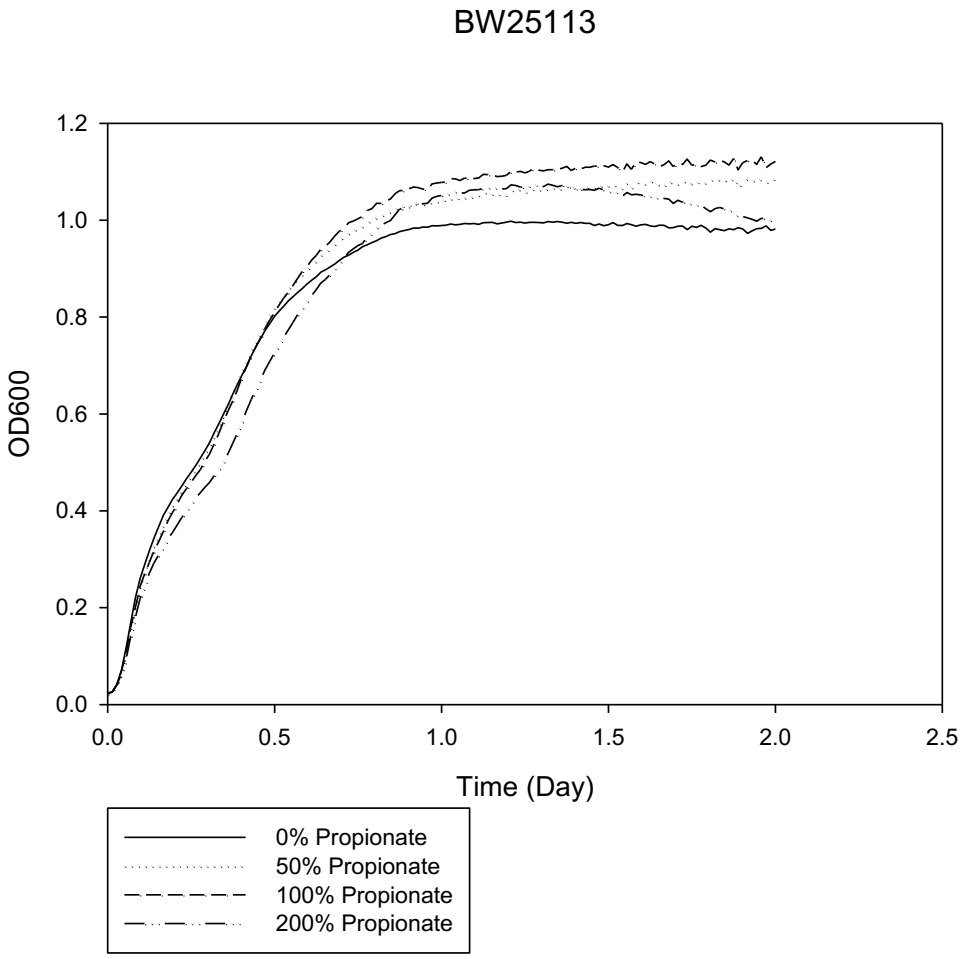

Figure 8. 10% BW25113 with Propionate. *E. coli* BW25113 increased growth with the addition of propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

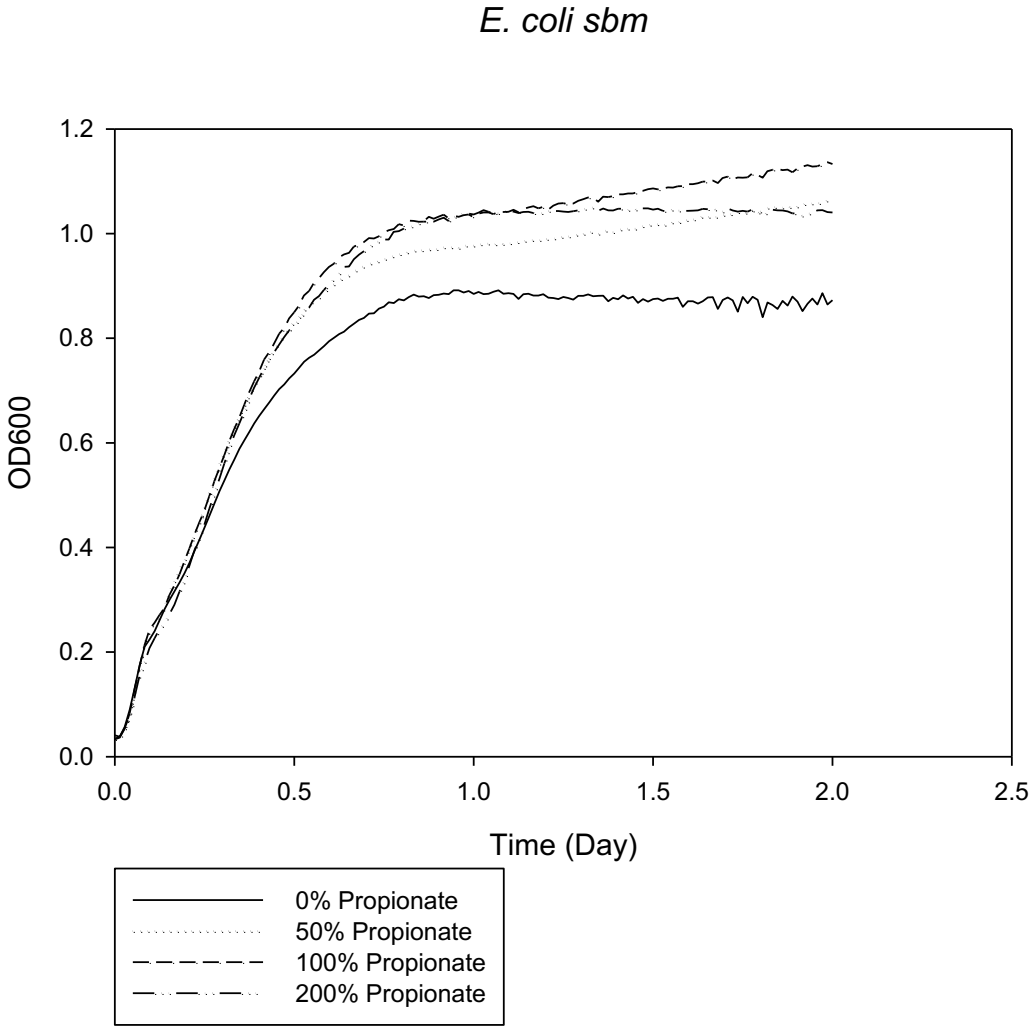

Figure 9. 10% *E. coli* mutant *sbm* with Propionate. *E. coli* mutant *sbm* increased growth with the addition of propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

10% Inoculum

BW25113 grew best with the addition of 0.9134mg/L, 1.8263mg/L, and 3.6526mg/L of propionate (Figure 8). The *E. coli* mutant showed increased fitness with the addition of propionate (Figure 9). E. faecalis showed optimal growth at 0mg/L, 0.9134mg/L, and 1.8263mg/L of propionate (Figure 10). The BW25113/*E. coli* mutant mixed culture showed increasing fitness with the increasing addition of propionate

(Figure 11). The *E. faecalis/E. coli* mutant did not show a significant difference between any of the levels of propionate (Figure 12). *E. feacalis* grew at a much lower density than the rest of the cultures, but showed optimal growth with 0.9134mg/L and 1.8263mg/L of propionate (Figure 13).

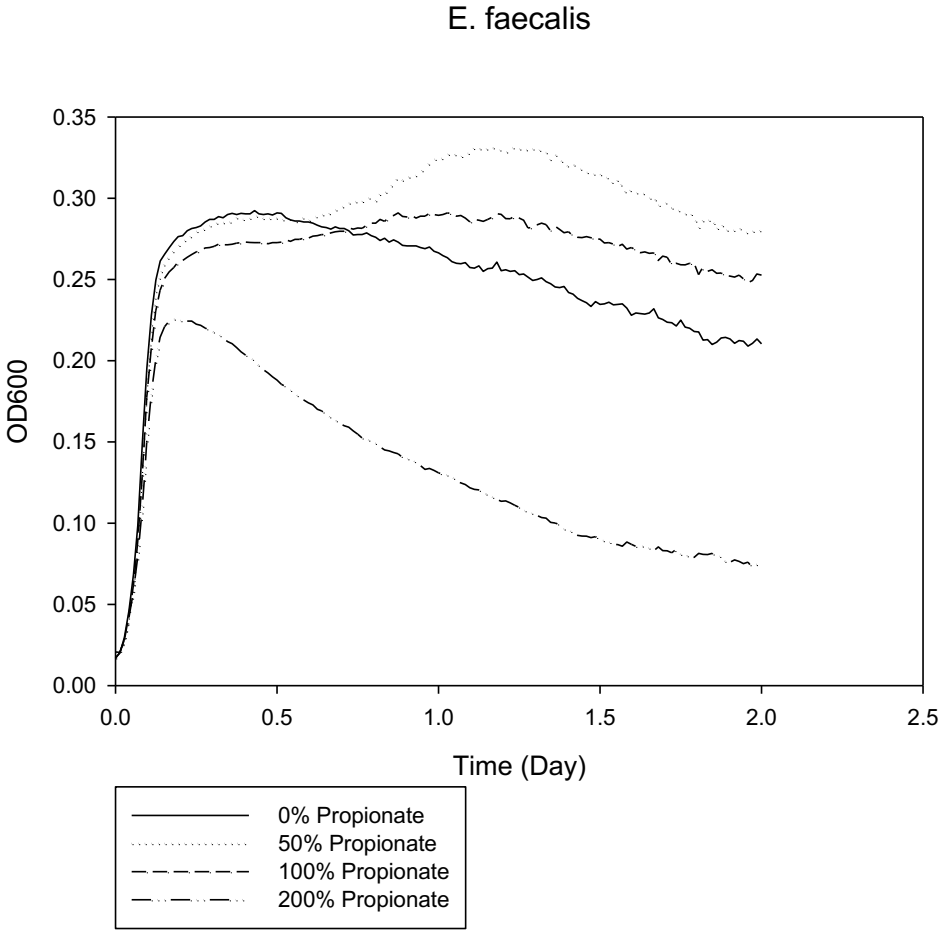

Figure 10. 10% *E. faecalis* with Propionate. *E. faecalis* showed optimal growth at 0mg/L, 0.9134mg/L, and 1.8263mg/L propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

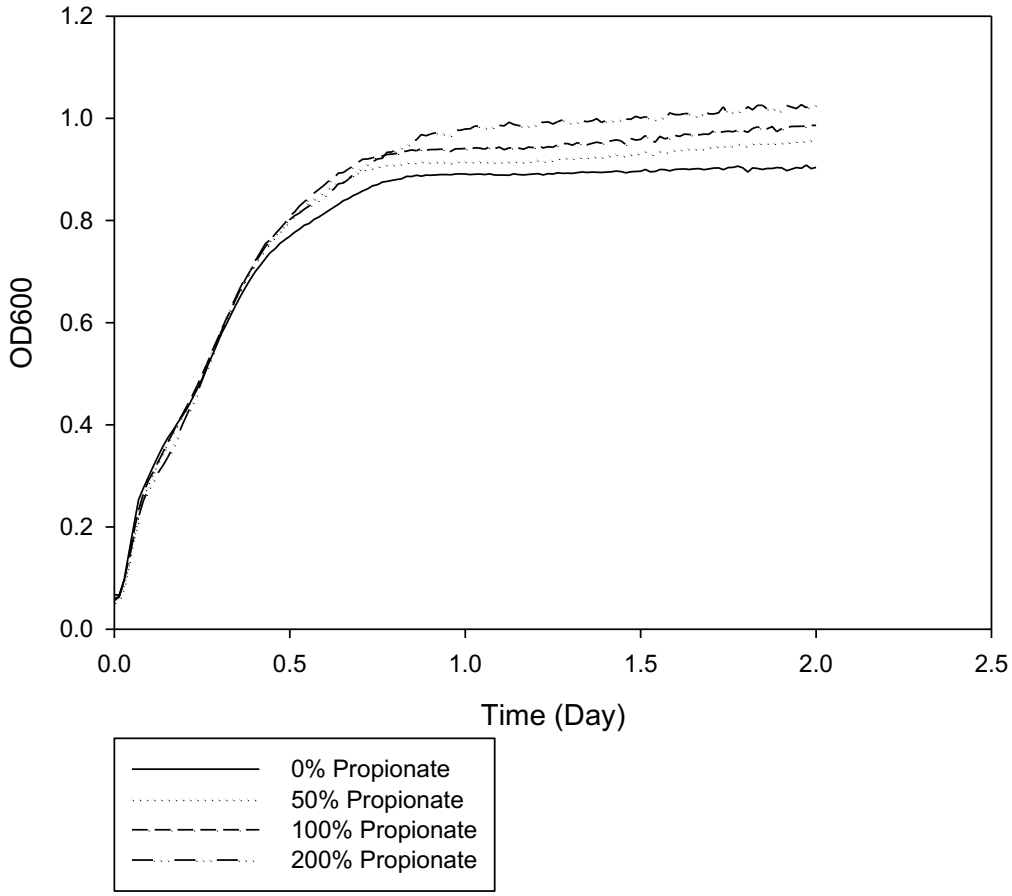

Figure 11. 10% BW25113/ *E. coli sbm* Mixed Culture with Propionate. Mixed culture *E. coli* BW25113/*E. coli* mutant *sbm* showed increasing growth with increasing propionate levels, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

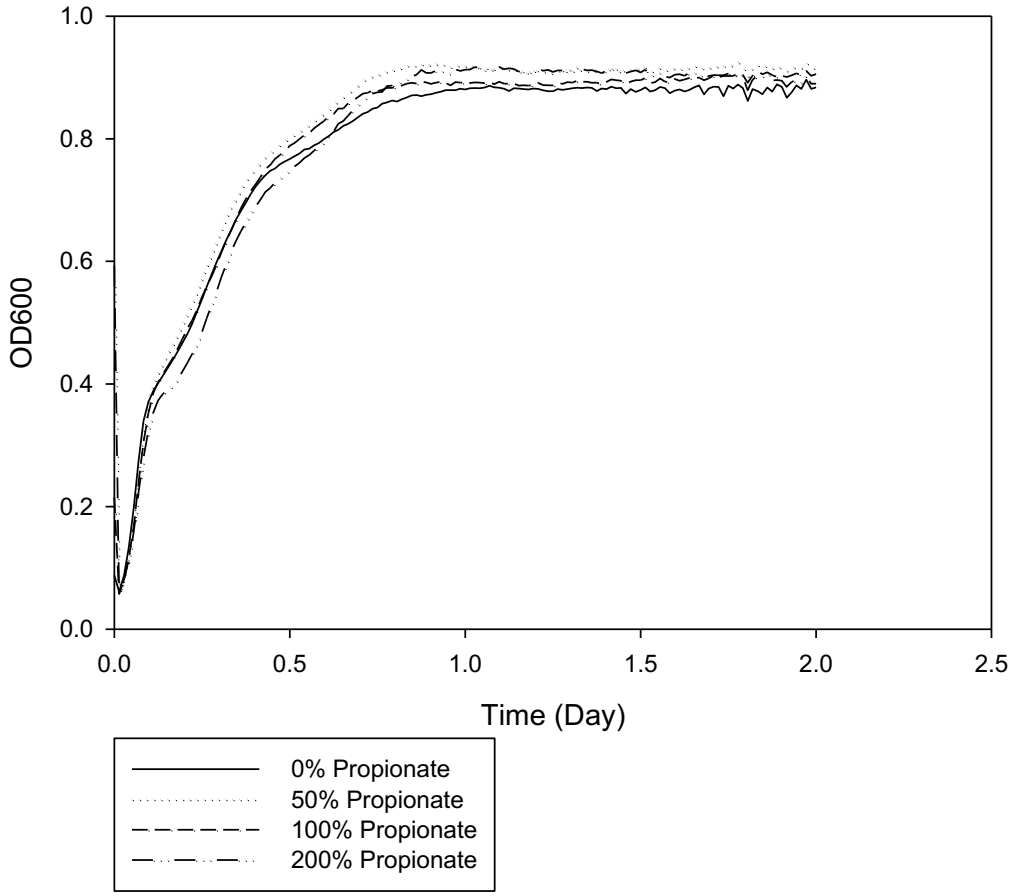

Figure 12. 10% *E. faecalis*/ *E. coli sbm* Mixed Culture with Propionate. Mixed culture *E. faecalis* /*E. coli* mutant *sbm* showed similar growth with all levels of propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

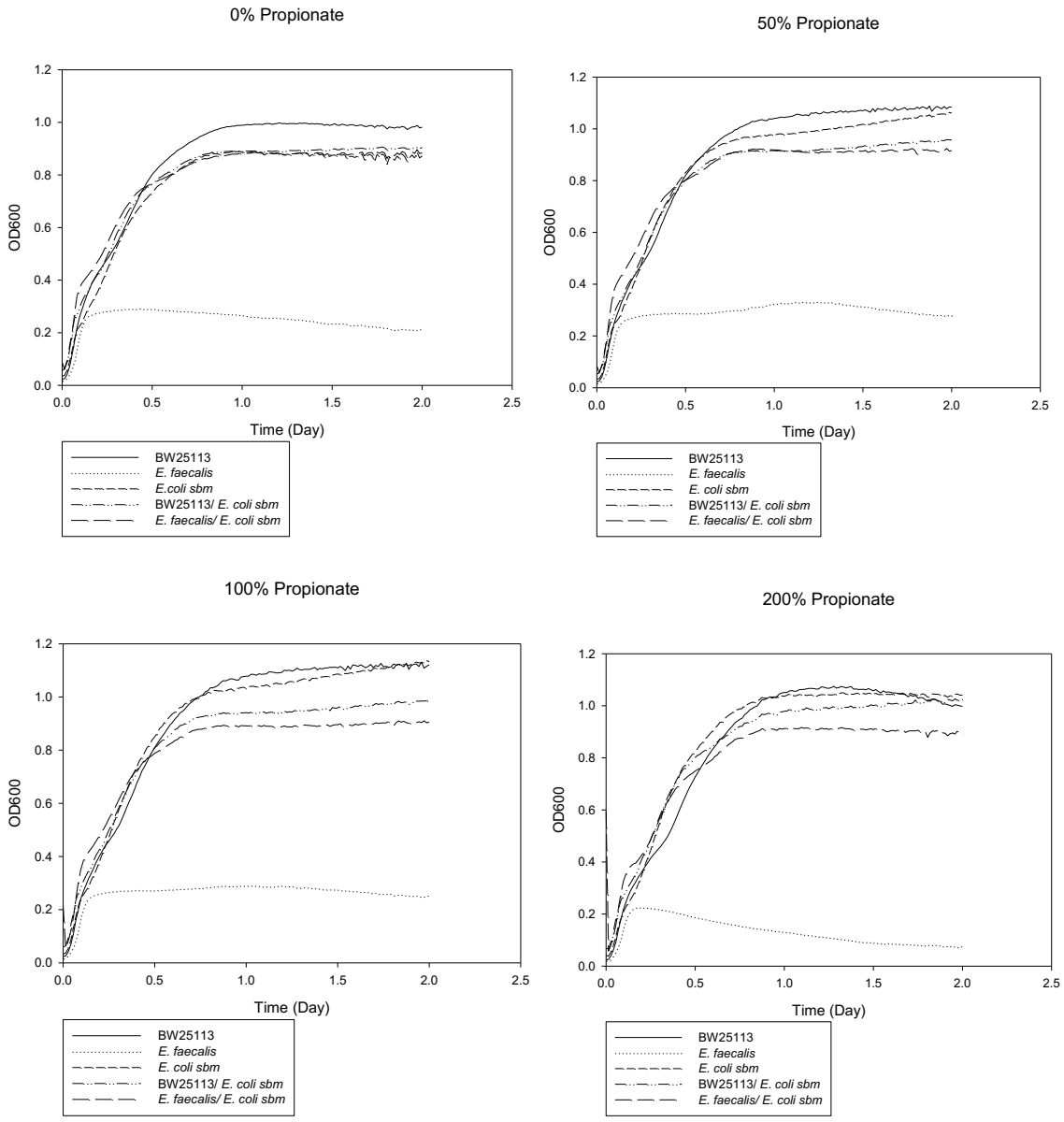

Figure 13. 10% Inoculum Growth within Propionate Levels. *E. faecalis* grew at much lower cell densities than the two *E. coli* strains. As propionate levels increased, growth of both *E. coli* strains increased. *E. faecalis* showed optimal growth with 0mg/L, 0.9134mg/L and 1.8263mg/L propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

1% Inoculum CFU Counts

At 1% of overall media, *E. faecalis* /*E. coli* mutant sbm grew better in mixed culture compared to pure culture at all levels of propionate. The mixed culture grew best with 3.6526mg/L of propionate. The mixed culture grew about the same with 0mg/L and 0.9134mg/L of propionate (Figure 14).

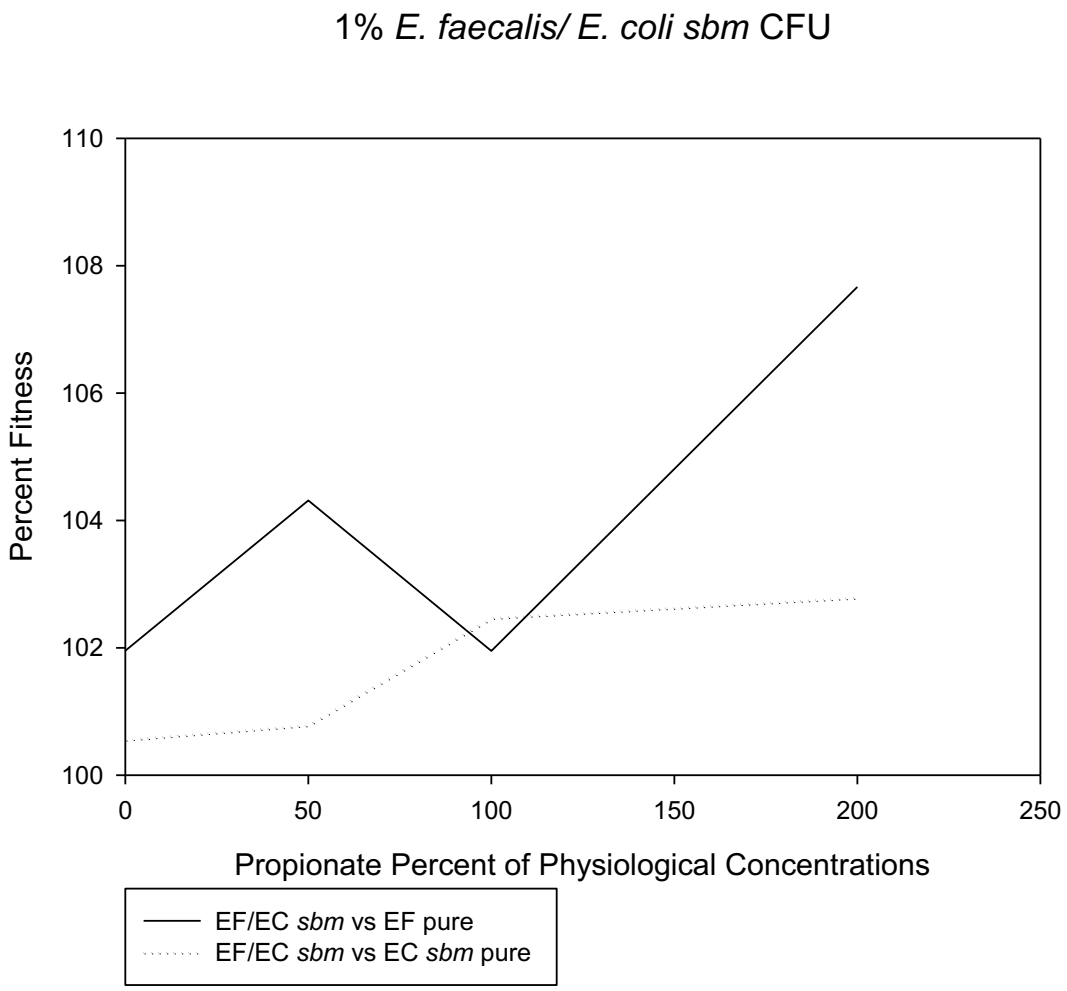

Figure 14. 1% Inoculum Mixed Culture Fitness CFU. *E. faecalis* /*E. coli* mutant sbm mixed culture grew better than the pure cultures and had optimal growth with 3.6526mg/L of propionate. The mixed culture showed about the same growth at 0mg/L and 1.8263mg/L of propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

10% Inoculum CFU Counts

At 10% of overall media, the *E. faecalis* /*E. coli* mutant sbm mixed culture grew better than the pure cultures at all levels of propionate. The mixed culture showed optimal growth with 0.9134/L of propionate and growth decreased with increased propionate addition.

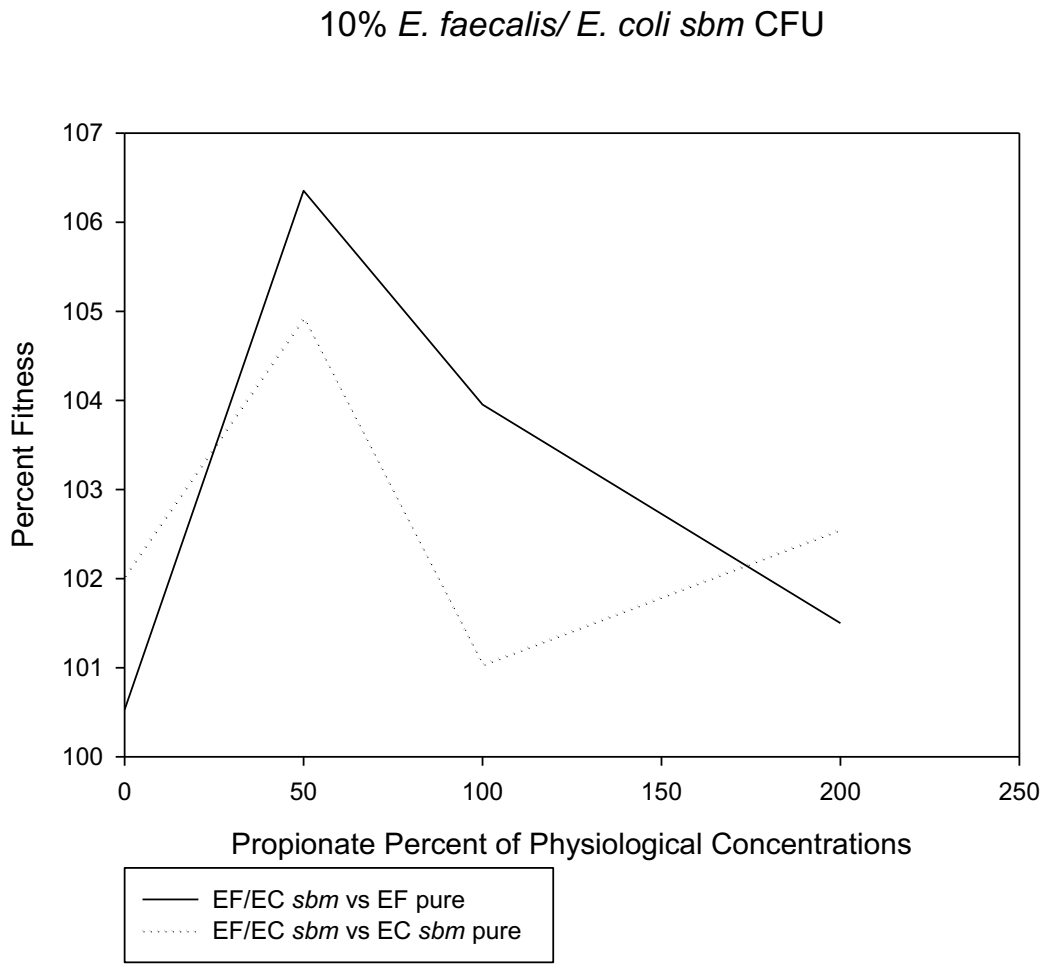

Figure 15. 10% Inoculum Mixed Culture Fitness. *E. faecalis*/*E. coli* mutant *sbm* mixed culture grew better than the pure cultures and showed optimal growth with 0.9134mg/L of propionate. Growth decreased with the increase of propionate, where 0% is 0mg/L, 50% is 0.9134, 100% is 1.8263mg/L, and 200% is 3.5626mg/L.

IV. DISCUSSION

E. coli and *E. faecalis* are able to coexist in the gut of many different species including humans. However, how they are able to coexist is still not well understood. Of the approximately 4,000 nonessential genes screened, one showed promise. Methylmalonoyl-CoA mutase may offer insight to better understanding how the two bacteria are able to co-inhabit. As part of the four gene operon, *sbm-ygfD-ygfG-ygfH,* Sbm, methylmalonoyl-CoA mutase, catalyzes the rearrangement of succinyl-CoA to L-methylmalonoyl-CoA. YgfD, whose function was previously unknown, has shown to have GTPase activity and that binding a non-hydrolyzable GTP analog stabilizes the association to *sbm in vitro* (Froese et al. 2009). YgfG, methylmalonoyl-CoA decarboxylase, catalyzes the decarboxylation of methylmalonoyl-CoA to produce propionoyl-CoA. YgfH, propionoyl-CoA: succinate-CoA transferase, transfers the CoA of propionoyl-CoA product to an available succinate, which readies another round of succinate to propionate decarboxylation (Haller et al. 2000). Without this nonessential gene, the *E. coli* mutant has decreased fitness when in mixed culture *in vitro* with *E. faecalis* under normal conditions (Figure 1).

Results observed proved to be a function of cell density. At 1% of the culture, the *E. coli* mutant showed to have increased growth when propionate was added back into the culture with the 1.8263mg/L of propionate being optimal. The *E. faecalis* grew at much lower densities than the *E. coli* in all conditions but showed increased fitness with 0.9134mg/L and 1.8263mg/L of propionate. When grown together in mixed culture, the cell density was between that of the two pure cultures but did not show a significant difference between the different amounts of propionate added.

At 10% of the culture, the *E. coli* mutant showed similar results to the lower bacterium inoculum percentage, increased fitness with the increased addition of propionate. The *E. faecalis* again, had lower cell density than the *E. coli* mutant but showed increased fitness with 0.9134mg/L and 1.8263mg/L of propionate. However, it had a significant decrease in fitness in 3.6526mg/L of propionate.

At both the 1% and 10% inoculum levels, the *E. faecalis* /*E. coli* mutant *sbm* mixed culture grew better than the pure cultures. The 1% inoculum showed best growth with 3.6526mg/L of propionate and close to the same growth with 0mg/L and 1.8263mg/L of propionate. The 10% inoculum showed optimal growth with 0.9134mg/L of propionate. The mixed culture growth decreased with the increased addition of propionate. These results demonstrate that the two bacteria grow better when together than they do alone in pure culture.

Short chain fatty acids are the major products of dietary carbohydrates in colonic bacterial fermentation. The leading compounds are acetic, propionic, and n-butyric acid (Hosseini et al. 2011). Butyrate is known as an energy source for colonocytes (Roediger 1980). Acetate is used for the synthesis of fatty acids and as a substrate for liver cholesterol (Hosseini et al. 2011). Propionic acid has been shown to have cholesterol lowering effects (Delzenne, Williams 2002). Suggesting that propionate supports a healthy system indicates that a diverse and steady equilibrium of bacteria is present. Bacteria utilize propionate as an energy source to maintain life. Therefore, an unhealthy, or unbalanced levels of propionate could lead to a disturbance of equilibrium among the bacterial community.

It is logical to believe that there is more occurring between *E. coli* and *E. faecalis*

that allows them to coexist in the gastrointestinal tract. Cell density could play a major role in how the bacteria compete. If one greatly outnumbers the other, nutrient availability and space become issues for the smaller bacterial population. The metabolite waste product may negatively affect the smaller population. There is still much to discover about how *E. coli* and *E. faecalis* are able to coexist and how to better treat them. Since this study was performed under aerobic conditions and did not include biofilm studies, future research in biofilm formation and anaerobic conditions would be beneficial.

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