CHARACTERIZATION OF CATION CHANNELS IN E. COLI

An Undergraduate Research Scholars Thesis

by

MADELINE PAIGE SMOOT

Submitted to the Undergraduate Research Scholars program
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. Steve Lockless

May 2016

Major: Microbiology

TABLE OF CONTENTS

ABSTRACT

Characterization of Cation Channels in *E. coli*

Madeline Paige Smoot
Department of Biology
Texas A&M University

Research Advisor: Dr. Steve Lockless
Department of Biology

Escherichia coli (*E. coli*) has a potassium (K^+) channel (*kch*) and a non-selective ion channel (*trkH/G*). The goal of this project is to explore the individual function and modes of regulation for each system, which will lead to a more thorough understanding of the role that K^+ homeostasis plays in bacterial physiology. Using phage transduction, we generated strains of *E. coli* that are deficient in each system. Growth and motility of mutant strains were assayed under varying conditions (physiological conditions, ion concentration, [Na^+/K^+], presence of other cations) and compared to that of wild-type *E. coli* to determine how K^+ transport systems impact other biological processes. Mutants were also assessed for change in antibiotic sensitivity as compared to the wild-type. Upon completion, the project will shed further light on prokaryotic ion transport systems.

ACKNOWLEDGMENTS

Participating in the Undergraduate Research Scholars program has been a tremendous learning experience. To Dr. Steve Lockless, thank you for the opportunity to work on the project. Additionally, I recognize Sarah Beagle for her assistance with my project and responding to a multitude of persistent inquiries. I would like to thank the members of the Lockless lab for their continued support and guidance throughout the duration of my time in the lab. I gratefully acknowledge Honors and Undergraduate Research for providing this program to the undergraduates at Texas A&M University. I extend gratitude to the Texas A&M Department of Biology for their assistance throughout my time at Texas A&M.

NOMENCLATURE

BW25113Δ*kch* - deletion of *kch,* a potassium channel

BW25113Δ*trkA* - deletion of t*rkA,* a regulatory component of the *Trk* cation uptake system

BW25113Δ*trkE* - deletion of *trkE,* a component of the *Trk* cation uptake system

BW25113Δ*trkG* - deletion of *trkG* channel, a component of the *Trk* cation uptake system

BW25113Δ*trkH* - deletion of *trkH* channel, a component of the *Trk* cation uptake system

MG1655Δ*kch* - deletion of *kch,* a potassium channel

MG1655Δ*trkA* - deletion of t*rkA,* a regulatory component of the *Trk* cation uptake system

MG1655Δ*trkE* - deletion of *trkE,* a component of the *Trk* cation uptake system

MG1655Δ*trkG* - deletion of *trkG* channel, the membrane portion of the *Trk* cation uptake system

MG1655Δ*trkH* - deletion of *trkH* channel, the membrane portion of the *Trk* cation uptake system

CHAPTER I

INTRODUCTION

Ion channels are integral membrane proteins that provide for the movement of charged ions across the cell membranes and down their electrochemical gradients.[1] This project will study the roles of the potassium-selective channel (*kch*) and the non-selective ion channel (*trkH/G*) of *E. coli*, a common prokaryotic model organism. Primarily, ion channels have been characterized for their role in eukaryotic cellular signaling via action potentials; however, a role for these proteins in prokaryotes has not yet been described.

Previous work has shown that *Trk* is a significant potassium (K^+) uptake system in *E. coli*. *E. coli also has* a K^+ channel, *kch*.[2] The *Trk* system has 3 primary components *(trkA, trkH, and trkG*). T*rkE* represents a fourth possible component, but its role is still controversial. The *Trk* system was previously thought to be a constitutive, low-affinity K^+ transporter, but recent work has shown that *trkH/A* constitute a non-selective ion channel, raising more questions about its function.[3,4] Lastly, the *Kch* channel is proposed to be involved in selective K^+ conduction, but it is unclear under what conditions the channel is active.[6]

Using genetics and physiological experiments, we will further characterize the role of bacterial ion channels. As K^+ is the major intracellular ion, characterizing the individual functions of these seemingly redundant systems will lead to a clearer understanding of role of K^+ and K^+ transport in bacterial physiology.

CHAPTER II

OBJECTIVES AND METHODOLOGY

E. coli maintains at least four K^+ uptake systems (*Kdp, Kup, Trk,* and *Kch*), but the function of the individual systems remains unclear. We focused on two of the systems - *Kch* and *Trk*. We hypothesized that each respective system serves to maintain cellular homeostasis under various stress conditions. We postulated that each system plays an individual role in affecting the motility behavior of the cell in comparison to the wild type cell. Furthermore, we anticipated that deletions of K^+ uptake systems would affect or change the sensitivity of *E. coli* to standard antibiotics.

Objective 1

Construct *E. coli* deficient potassium uptake mutants (Δ*trk*A, Δ*trkE*, Δ*trkH*, Δ*trkG*, and Δ*kch*) in the MG1655 *E. coli* background strain, and establish a stock of each strain for the laboratory in the strain library.

Methods

Bacteriophage (P1) transduction was employed to move single gene deletions between strains.[8] Phage was grown up on the donor strain (BW25113) that had the desired single deletion that needed to be moved into a recipient strain background (MG1655). The donor strain phage lysate was then collected and used to infect the desired recipient strain. *E. coli* MG1655 cells that were not infected by P1 phage were used as the negative control. Any positive transductants grew in the presence of kanamycin as the resistance marker was gained during infection. The strains

were sequenced to confirm clean gene knock outs after construction. Any strains that showed a clean single deletion were maintained in the strain stock.

Objective 2

Complete growth assays to determine the importance of ion channels under physiological conditions.

Methods

Growth of a mutant strain was assayed in rich growth media. *E. coli* MG1655 was employed as the wild-type control to which the mutant's growth was compared to. An overnight liquid culture was started by inoculation with a single colony of *E. coli*. The overnight culture was then brought down to an optical density value of 0.1 when measured at 600 nm. The culture was then measured every quarter hour, and was allowed to grow until the sinusoidal growth curve reached saturation.

Objective 3

Establish if the deletion of potassium channel or transport systems affect the ability of the bacteria to exhibit swimming motility. Establish if the deletion causes a distinct motility phenotype. Determine what effect the addition of various cations will have on motility.

Methods

Motility assays were conducted for each of the strains using a motility growth medium.[9,10] The motility medium was inoculated with either a mutant or wild-type strain and was incubated for

14 hours. Two different inoculation methods were employed – stabbing with a single isolated colony or pipetting one microliter of suspended *E. coli* cells at an OD_{600} of 0.1. Growth and motility of the mutant strains were compared to their respective parental *E. coli* strain (either MG1655 or BW25113). The standard motility media is LB and all plates contained standard concentrations of yeast extract (5 grams/liter) and tryptone (10 grams/liter). However, the concentration of ions (potassium or sodium) and agar were altered depending on the experiment. Deviations in sodium and potassium salt concentrations from the standard motility media were tested. Other conditions (agar concentration, temperature, presence of divalent cations) within the motility plates were also tested.

Objective 4

Establish if a single deletion of the cryptic potassium channel, *Kch*, will affect the sensitivity of the bacteria to standard antibiotic concentrations of different antibiotics (Kanamycin, Chloramphenicol, or Ampicillin).

Methods

A bacterial lawn of the BW25113Δ*kch* strain was spread across a standard LB agar plate. The lawn was allowed to dry and set on the plate for several minutes. During this period, sterile paper filter disks were inoculated with 20 µL of chloramphenicol (100 mg/mL), ampicillin (25 mg/mL), or kanamycin (50 mg/mL). The disks were then uniformly distributed on the plate. The plates were incubated overnight at 37°C for 14-16 hours. The diameters of inhibition of growth caused by each antibiotic were measured and plates were photographed.

CHAPTER III

RESULTS

A total of four single mutations were moved from the BW25113 background to the MG1655 background via generalized phage transduction. The mutations moved were single deletions of each of the respective system components of the *trk* potassium transport system - *trkA*, *trkG*, *trkH*, and *trkE*. In addition, the antibiotic resistance markers were removed from two of the four strains (BW25113Δ*trkE* and BW25113Δ*trkG*). All strains were maintained in the laboratory strain collection stock.

BW25113Δ*kch* produces a concentric banding pattern (shown in Figure 1) when grown on motility media plates. This motility phenotype was novel and thus it was necessary to identify the optimal motility assays conditions to visualize the phenotype and obtain qualitative data.

Two different concentrations of salt were tested - 0.14 M and 0.17 M. In addition, two distinct salts were tested - NaCl and KCl. It was seen that as the potassium concentration increased, the total distance swam was larger and the spacing between bands increased (Figure 1). Additionally, sodium as the primary cation in the media yielded narrower banding than potassium (Figure 1).

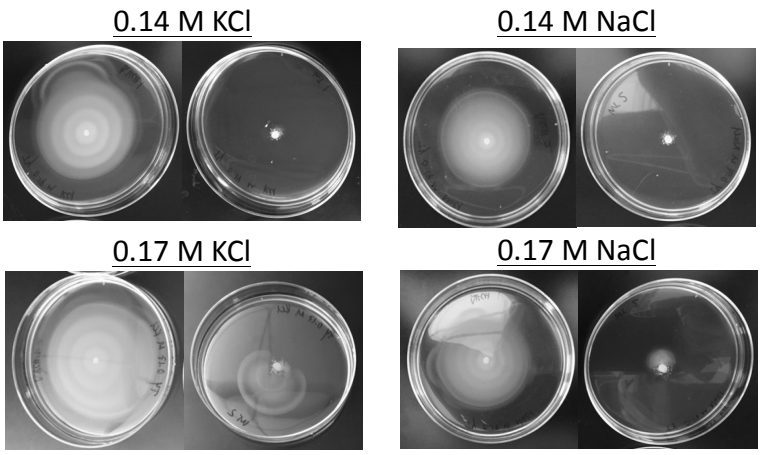


Figure 1. Motility plates (LB media, 0.22% agar) containing 0.14 M or 0.17 M NaCl or KCl were inoculated with 1 μL of BW25113Δ*kch* (left) or wild-type BW25113 (right) and incubated for 14 hours at 37°C. It was found that as salt concentration increased, the banding became more defined. Sodium produced narrower bands than potassium.

The effect of unique inoculating methods on the motility phenotype was assessed next. The two methods tested were pipetting and stabbing; pipetting is slow but able to place a specific number of cells on the surface of the plate, while stabbing is quicker but an undefined number of cells are distributed through the thickness of the plate. For pipette inoculations of the motility plates, cultures were grown to early stationary phase and diluted down to an OD_{600} of 0.1. The pipette was then held over the center of the plate and 1 μL of inoculum was released on the surface. The second inoculation method tested was the stabbing method. In this method, a single colony from a plate is picked using a sterile toothpick and stabbed into the motility plate. It was found that when either method was employed, strains demonstrated the same consistent phenotype (Figure 2).

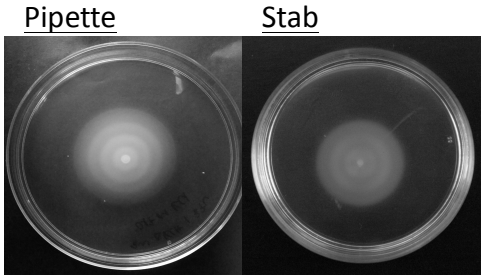


Figure 2. A comparison of the pipetting (left) versus stabbing (right) inoculation method. In the pipetting method, 1 μL of BW25113Δ*kch* culture is pipetted directly into the center of the motility plate. In the stab method, a single BW25113Δ*kch* colony is picked using a sterile toothpick and then stabbed into the center of the motility plate. There was no affect on phenotype presentation of BW25113Δ*kch* when using either method. This comparison was performed using motility media (LB media with 0.17 M KCl, 0.25% agar). Plates were incubated for 14 hours at 37°C.

The last optimization of the motility assay was the agar concentration of the motility plates. After testing two agar concentrations (0.22% or 0.25%), a 0.25% agar concentration was chosen for future experiments (Figure 3). The banding was not eliminated by the higher agar concentrations, but was logistically simpler to work with; a higher concentration was not used because *E.coli* switches from swimming to swarming behavior around 0.3% agar.

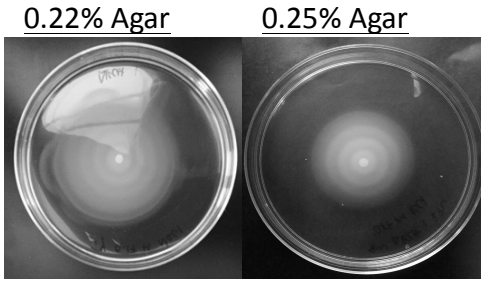


Figure 3. A comparison of the effects of 0.22% (left) or 0.25% (right) agar concentration on the motility phenotype of BW25113Δ*kch.* Altering the agar concentration did not significantly impact the phenotype. However, it was much simpler from an experimental standpoint to work with 0.25% agar. Thus, 0.25% agar was selected as the condition moving forward in motility assay work. The motility plates used in the comparison were LB 0.17 M KCl.

Temperature effects on motility were also assessed (Figure 4). The BW25113 wild-type exhibited growth only at the inoculum site at all 4 temperatures (30°C, 32°C, 35°C, and 37°C).

BW25113Δ*kch* was motile but had no bands at 30°C. Bands began to appear for BW25113Δ*kch* at 32°C and became more narrow and defined as the temperature increased from 32°C to 35°C to 37°C.

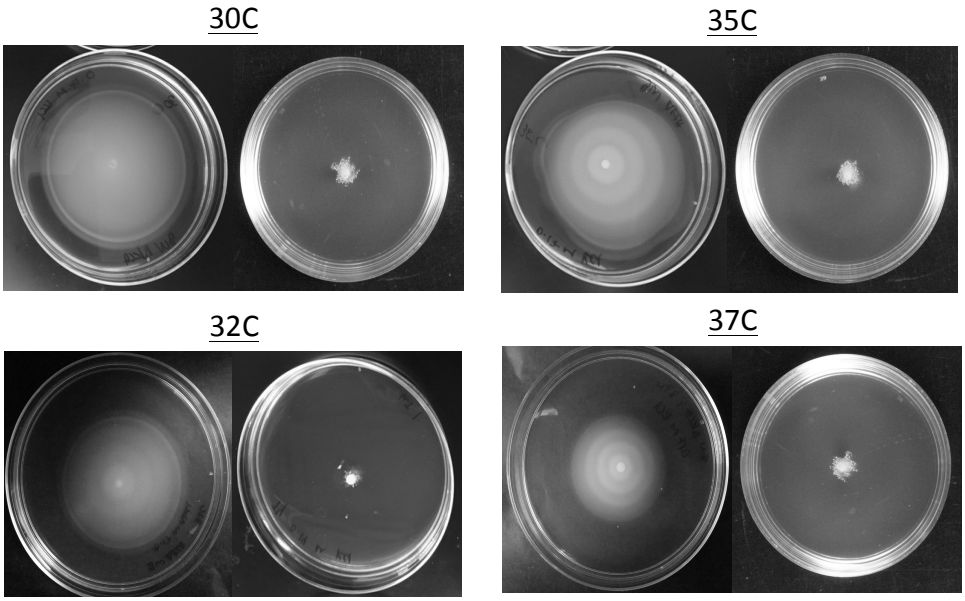


Figure 4. BW25113 Δ*kch* (left) and BW25113 wild-type (right) were tested for differences in motile behavior due to temperature. Both strains were tested using LB 0.17 M KCl, 0.25% agar motility plates that were incubated for 14 hours. The plates were incubated at 30°C, 32°C, 35°C or 37°C.

Other strains deficient in components of the *Trk* non-selective ion channel, were tested for their motility phenotype. The tests were under the following conditions: standard motility media with 0.17 M KCl and 0.25% agar, incubated at 37°C for 14 hours. BW25113Δ*trkE* and BW25113Δ*trkG* exhibited a motile banding pattern behavior while BW25113Δ*trkH* was non-motile and only grew at the inoculum site (Figure 5).

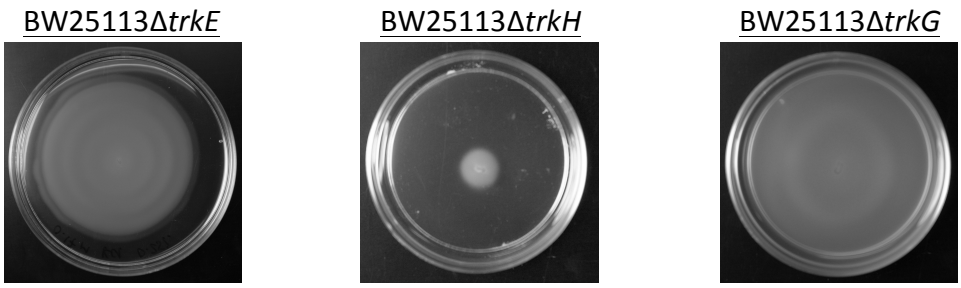
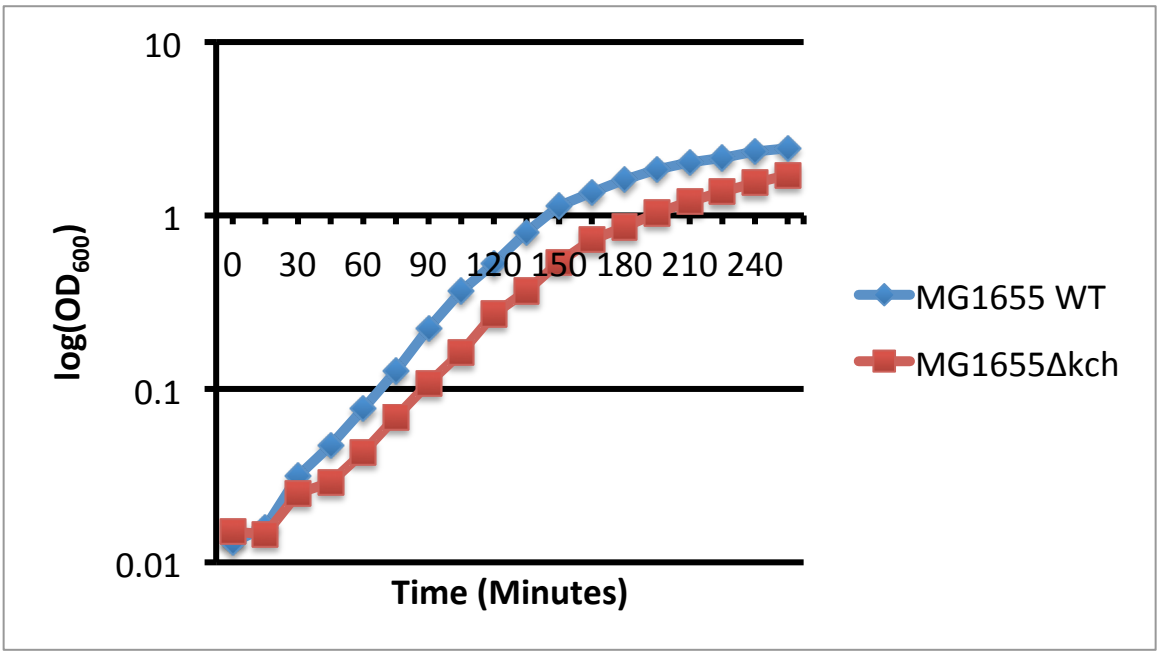


Figure 5. Other single deletion mutants deficient for components of the non-selective ion channel (*trk*) were analyzed for motile behavior. From left to right: BW25113Δ*trkE*, BW25113Δ*trkH*, and BW25113Δ*trkG*. LB 0.17 M KCl, 0.25% agar motility plates were inoculated with the respective strain and incubated for 14 hours at 37°C.

Previous work has shown that in liquid culture, motile strains exhibit growth defects in comparison to strains that are non-motile.[11] An *E. coli* MG1655 mutant deficient for the potassium channel, *kch*, and the parental MG1655 strain were assessed for growth in liquid culture. Cultures were incubated at 37°C with shaking. A comparison of the generation times revealed that the single deletion (MG1655Δ*kch*) exhibits a growth defect in liquid culture when compared to wild-type MG1655. The results are shown graphically in Figure 6 below.



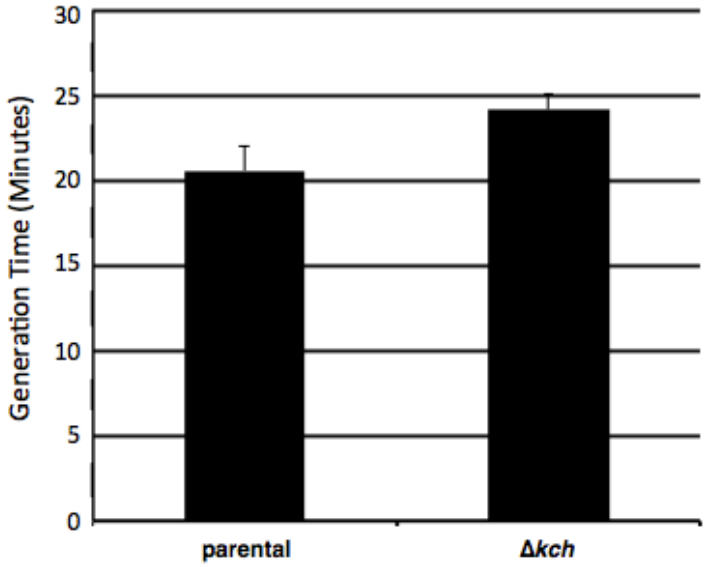


Figure 6. The above curve shows growth assays that were conducted for MG1655 parental and MG1655Δ*kch*. All strains were grown in LB broth aerobically at 37°C. Measurements of the OD_{600} value were taken every 15 minutes. MG1655Δ*kch* exhibits a growth defect compared to the parental strain in liquid culture.

Further investigation led us to ask if we could restore the wild-type motility phenotype (no banding) in the BW25113Δ*kch* background. Prior work in the channel field has shown that cesium (Cs^+) and barium (Ba^{2+}), other cations, can act as channel blockers that compete for binding of the channel with any potassium that may be present.[12] Thus, based on this previous work, Ba^{2+} and Cs^+ were added to the standard motility media (0.17 M salt, 0.25% agar) at a 10 mM concentration and phenotypic effects were recorded (Figure 7). It was anticipated that exposure to other cations would induce the concentric banding motility pattern in the wild-type BW25113 strain. This was not the case as seen in figure 7. Upon exposure to 10 mM barium, there was a reduction in the number of bands formed by BW25113Δ*kch,* and the bands were less defined.

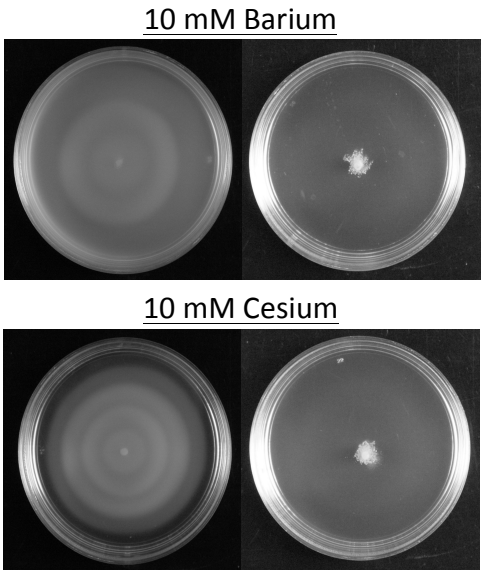


Figure 7.Test for induction of WT phenotype by other cations. Cations (either barium or cesium) were added at 10 mM concentrations to the motility media (LB 0.17 M KCl, 0.25% agar). It was hypothesized that blocking of uptake systems would induce the concentric band formation in wild-type BW25113. The preliminary results show that BW25113 wild-type (right) doesn't show the same phenotype as BW25113Δ*kch* (left) after exposure to 10 mM cation concentration. In addition, it should be noted that the presence of barium yields a reduction in the number of bands and an increase in width of bands for BW25113Δ*kch*.

Lastly, antibiotic sensitivity of wild-type BW25113 and BW25113Δ*kch* strains was tested (Figure 8). The BW25113 wild-type strain was more sensitive to ampicillin and chloramphenicol than the channel deletion strain (BW25113Δ*kch*). Both exhibited similar sensitivity to kanamycin.

Antibiotic (mg/mL)	Diameter of Zone of Inhibition for WT (Plate 1, Plate 2) (N=2)	Diameter of Zone of Inhibition for Δ*kch* (Plate 1, Plate 2) (N=2)
Kanamycin (50 mg/mL, 20 µL)	3.0 cm, 2.9 cm	2.8 cm, 2.8 cm
Ampicillin (25 mg/mL, 20 µL)	2.6 cm, 2.5 cm	1.2 cm, 1.2 cm
Chloramphenicol (100 mg/mL, 20 µL)	2.9 cm, 3.0 cm	1.5 cm, 1.4 cm

Figure 8. Antibiotic sensitivity was tested for BW25113Δ*kch* and compared directly to the sensitivity of BW25113 wild-type. The table summarizes the diameters of the zone of inhibition for all three antibiotics for both wild-type and Δ*kch*. BW25113Δ*kch* is clearly more resistant than BW25113 wild-type to both ampicillin and chloramphenicol.

CHAPTER IV

CONCLUSION

In summary, BW25113Δ*kch* moves in a concentric banding pattern in motility media. BW25113Δ*trkG* and BW25113Δ*trkE* demonstrated variations of the same motility phenotype. Increasing salt concentrations caused the bands to be more distinct. Bands were still present even when BW25113Δ*kch* was exposed to other cations (barium or cesium). As temperatures increased, banding produced by BW25113Δ*kch* became more defined.

More work will need to be conducted in order to elucidate the underlying mechanism of the banding pattern formation caused by deletions of several different channel components. We have proposed one model to describe the motility phenotype (Figure 9). The model is that cells deplete nutrient(s) in the media at the inoculation site and swim outwards from the inoculation site to find a new environment. Cells then reach a point X where there are more nutrients, slow down their motility, and divide. Point X then becomes starved of nutrients and the cells swim outward to stop at point Y when nutrients are more abundant. This cycle is then repeated.

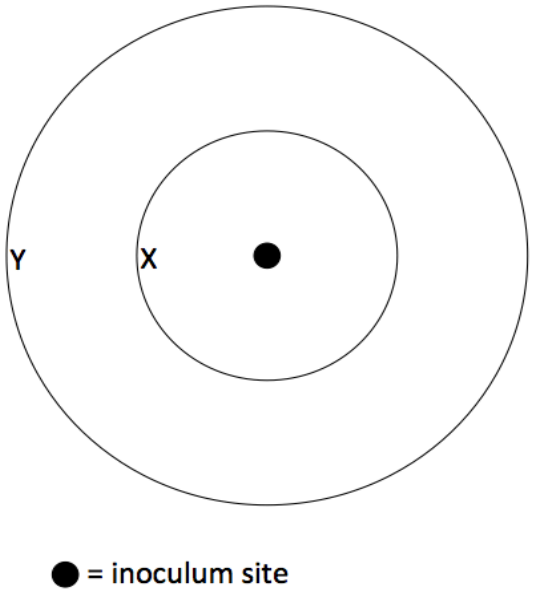


Figure 9. A depiction of the model for the banding motility phenotype.

In order to further understand the potential model described, a time lapse video of the cellular motility could be taken of all strains that exhibit the phenotype.

Future directions include generating double and triple system-deficient strains. If the cells are able to survive a deficiency in two or three systems, then the cells will be analyzed for growth deficiencies, motility phenotypes, and sensitivity to antibiotics. It is expected that double and triple deficient systems will either show similar phenotypes or not be viable at all. In order to determine essential systems for the phenotype, rescue assays should be performed. Rescue assays will reintroduce one of the deleted proteins. Based on the results, one will be able to determine if that particular reintroduced protein is essential for exhibition of the phenotype by the cell. This will lead to more understanding of how each system contributes to generation of the phenotype. Further data may allow for construction of an interaction map showing the role of each system in the cascade toward production of the phenotype.

REFERENCES

1. Kubalski, A. and Koprowski, P. (2001). Bacterial ion channels and their eukaryotic homologues. BioEssays 23:1148-1158.

2. Epstein, W. (2003). Progress in Nucleic Acid Research & Molecular Biology, vol. 75, pp. 293–320. Academic Press Inc., San Diego.

3. Kuo, M. (2005). Prokaryotic K^+ channels: From crystal structures to diversity. FEMS Microbiology Reviews 29:961-985.

4. Zhou, M. (2013). Gating of the TrkH ion channel by its associated RCK protein TrkA. Nature 496:317-322.

5. Trchounian, A. and Kobayashi, H. (1999). Kup is the major K^+ uptake system in Escherichia coli upon hyper-osmotic stress at a low pH. FEBS Letters 447:144–148.

6. Sivaprasadarao, A. (2002). Functional Properties of Kch, a prokaryotic homologue of eukaryotic potassium channels. Biochemical and Biophysical Research Communications 207:10-16.

7. Datsenko, K. (2000). One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. PNAS 97:6640-6645.

8. Miller, J. (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory Press.

9. Fall, R. (2003). Rapid Surface Motility in Bacillus subtilis Is Dependent on Extracellular Surfactin and Potassium Ion. Journal of Bacteriology 185:5627-5631.

10. Fall, R. (2005). Genetic Requirements for Potassium Ion-Dependent Colony Spreading in Bacillus subtilis. Journal of Bacteriology 187:8462-8469.

11. Prüss BM, Matsumura P. A regulator of the flagellar regulon of Escherichia coli, flhD, also affects cell division. *Journal of Bacteriology*. 1996;178(3):668-674.

12. Piasta KN, Theobald DL, Miller C. Potassium-selective block of barium permeation through single KcsA channels. *The Journal of General Physiology*. 2011;138(4):421-436. doi:10.1085/jgp.201110684.