Studies on Helicobacter Pylori motility: influence of cell morphology, medium rheology, and swimming mechanism

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STUDIES ON *Heliocobacter pylori* MOTILITY: INFLUENCE OF
CELL MORPHOLOGY, MEDIUM RHEOLOGY, AND SWIMMING
MECHANISM

by

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For my wife.
STUDIES ON HELICOBACTER PYLORI MOTILITY: INFLUENCE OF CELL MORPHOLOGY, MEDIUM RHEOLOGY, AND SWIMMING MECHANISM

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ABSTRACT

In this thesis, I present a detailed analysis of the role cell morphology, solution rheology, and swimming mechanism has on the motility of Helicobacter Pylori. H. Pylori, the bacterium that causes gastric ulcers, has a helical cell shape that has long been believed to provide an advantage in penetrating the viscous mucus layer protecting the stomach lining, its niche environment. I present results obtained by performing optical microscopic live cell bacteria tracking of wild-type H. Pylori and cell shape and flagella mutants of H. Pylori. Bacteria tracking experiments show that helical shaped bacteria swim faster than straight rod-shaped bacteria, and bacteria with larger number of flagella swim faster. Altering cell shape is found to have a smaller effect on swimming speed than altering the number of flagella a bacterium has. These experimental observations are then compared to resistive force theory predictions. Resistive force theory shows qualitative agreement to our experimental observations, but overestimates the increase in swimming speed for a helical cell when compared to straight rod cell. In addition to effect of cell morphology on motility, I explore how motility is altered in different polymer environments by tracking bacteria in pig gastric mucin, methylcellulose, and gelatin solutions and gels. Bacteria are found to increase their swimming speed non-monotonically with increasing polymer concentration, while the number of mobile bacteria is found to decrease with increased polymer concentration. I also present an analysis of the swimming mechanism used by H. Pylori. H. Pylori is found to
use a run-reverse swimming mechanism which I model as a random walk. This random walk model fits well to the experimental data and provides a theoretical tool for interpreting \textit{H. Pylori}'s swimming mechanism. Taken together these results provide a detailed description of the motility of \textit{H. Pylori} in different media and are applicable to the broad question of how \textit{H. pylori} infects and colonizes the mucus layer of the stomach.
## Contents

1 Introduction

1.1 Motivation and Background ........................................ 1

1.2 *Helicobacter Pylori* ............................................. 2

1.2.1 Discovery and Health Implications ............................. 3

1.2.2 Colonization of the Human Stomach ........................... 4

1.2.3 Motility and Morphology ........................................ 5

1.3 Bacterial Swimming .................................................. 7

1.3.1 The Hydrodynamics of Bacterial Swimming ................. 8

1.3.2 Bacterial Swimming Strategies and Chemotaxis ............ 11

2 Materials and Methods ............................................... 13

2.1 Bacteria Strains and Culturing ................................... 13

2.2 Microscopy and Particle Tracking ............................... 15

2.2.1 Phase Contrast Microscopy ..................................... 16

2.2.2 Fluorescence Microscopy ....................................... 17

2.2.3 Particle Tracking ................................................ 18

2.3 Trajectory Analysis .................................................. 20

2.3.1 Bacteria Trajectory Analysis .................................. 21

2.3.2 Microrheology ..................................................... 24

3 Experiments on the role of Cell Morphology on Motility ....... 27

3.1 Introduction .......................................................... 27
3.2 Materials and Methods ...................................................... 28
  3.2.1 Bacteria Strains and Culturing ....................................... 28
  3.2.2 Morphology Analysis .................................................. 28
  3.2.3 Preparation of Polymer Solutions .................................... 29
  3.2.4 Particle-tracking Microrheology ...................................... 30
  3.2.5 Bacteria Motility Measurements ...................................... 30
  3.2.6 Statistical Comparisons ............................................. 32
3.3 Results and Discussion .................................................. 33
  3.3.1 Microrheology of Polymer Solutions ................................ 33
  3.3.2 Morphological Measurements and Analysis ......................... 35
  3.3.3 Motility of *H. pylori* .............................................. 39
3.4 Conclusions ............................................................... 55

4 Restive Force Theory Model of Swimming ............................. 60
  4.1 Introduction .............................................................. 60
  4.2 Methods ................................................................. 60
  4.3 Results and Discussion ................................................ 64
    4.3.1 RFT Predictions for Helical Bacteria ............................ 64
    4.3.2 RFT Predictions for Straight Rod Bacteria ...................... 67
  4.4 Conclusions ............................................................ 70

5 Analysis of Reversal Swimming Mechanism ........................... 74
  5.1 Introduction .............................................................. 74
  5.2 Materials and Methods ................................................ 75
  5.3 Results and Discussion ................................................ 76
    5.3.1 Analysis of Run-Reverse Trajectories in Broth ............... 76
    5.3.2 Analysis of Run-Reverse Trajectories in Pig Gastric Mucin .... 79
    5.3.3 Analysis of Run-Reverse Trajectories in Methylcellulose ...... 81
    5.3.4 Theoretical Model of Run-Reverse Swimming .................... 82
List of Tables

3.1 Average cell shape parameters for each wild-type strain. [Martínez et al., 2016] 36

3.2 Table showing for each strain the: number of bacteria tracked, \( n \), average swimming speed, \( v_{\text{avg}} \), median swimming speed, \( v_{\text{m}} \), maximum swimming speed measured, \( v_{\text{max}} \), and standard deviation of the swimming speeds, \( \sigma \). [Martínez et al., 2016] 42

3.3 Table for B128 and its flagella mutants showing: number of bacteria tracked, \( n \), average swimming speed, \( v_{\text{avg}} \), median swimming speed, \( v_{\text{m}} \), maximum swimming speed measured, \( v_{\text{max}} \), standard deviation of the swimming speeds, \( \sigma \), average percent track linearity, \( \% \) TL, and fraction of immobile bacteria, \( \% \) Immobile. [Martínez et al., 2016] 53

4.1 Median speeds in PGM 15 mg mL\(^{-1}\), shape factor (\( S_h \)), and the product of \( S_h \) and median flagellum number (\( S_h N_f \)). \( S_h \) and \( S_h N_f \) are in units of \( 10^3 \) \( \mu \text{m sec}^{-1} / \text{pN} \mu \text{m} \) [Martínez et al., 2016]. 66

4.2 Median speeds in PGM 15 mg mL\(^{-1}\), shape factor (\( S_h \)), and estimated torque (\( T_m = v_{\text{m}}/S_h \)) for LSH100 and PMSS1 and their respective csd6 mutants. \( S_h \) is in units of \( \mu \text{m sec}^{-1} / \text{pN} \mu \text{m} \) [Martínez et al., 2016]. 68

4.3 Drag coefficients for wild-type LSH100 and PMSS1 and their \( \Delta \)csd6 mutants. \( \alpha \) is in units of \( \text{pN}/(\mu \text{m sec}^{-1}) \), \( \beta \) and \( \gamma \) are in units of \( \text{pN} \mu \text{m sec} \). 70

5.1 Parameters obtained by fitting the \( t_{\text{run}} \) distribution with a gamma function
\[
P(t, a, \lambda) = \lambda^a t^{a-1} e^{-\lambda t} / \Gamma(a).
\]
80
5.2 Rotational diffusion constant of *H. pylori* in BB10, PGM 15 mg mL\(^{-1}\), and PGM 30 mg mL\(^{-1}\) obtained by fitting \(\langle \hat{e}(t) \cdot \hat{e}(0) \rangle\) to equation 5.3.
List of Figures

1.1 TEM image of *Helicobacter Pylori*. Scale bar indicates 1 µm ............... 3
1.2 Diagram of *Helicobacter Pylori* infection. [Y.tambe, ] ..................... 4
1.3 Sketch of run-tumble (A), run-reverse (B), and run-reverse-flick (C). Image taken from Taktikos et al. [Taktikos et al., 2013] ......................... 12

2.1 Diagram from Sycuro et al., 2010 illustrating how Csd proteins cause *Helicobacter Pylori*’s helical cell shape. [Sycuro et al., 2010] ............... 14
2.2 Phase contrast image of a *Helicobacter Pylori* bacterium obtained using a 100x objective and CMOS camera. .................................................. 17
2.3 Image depicting 3 µm fluorescent beads imaged using a fluorescence microscope with a 20X objective and captured using a CMOS camera. ................. 18
2.4 Trajectory of *H. Pylori* swimming in broth media along with plots of speed \(v\) and absolute angle change \(|\Delta \phi|\) as functions of time. Red circles indicate times identified to be reorientation events. ........................................ 22
3.1 Mean square displacement (MSD, µm²) values as a function of time (sec) for 1 µm fluorescent particles (A) and LSH100 ΔmotB bacteria (B) in broth (BB10), PGM (15 mg mL⁻¹ and 30 mg mL⁻¹) and MC (10 mg mL⁻¹ and 15 mg mL⁻¹) along with (C) Average power of law exponents (α). Average viscosity values (η, cP) and their standard deviations are summarized in the table above (see text for calculation details). All error bars represent standard error and dashed lines represent linear scaling of MSD over time (α=1). *This value is significantly larger compared to the others due to the gel-like nature of PGM at 30 mg mL⁻¹. [Martínez et al., 2016] . . . . . . . . 34

3.2 (A) Side curvature vs. cell length (µm) for individual bacterial cells LSH100 (blue, n=282), B128 (green, n=274), and PMSS1 (orange, n=222) bacteria. Inset panel: TEM images of each. Scale bar = 1 µm. (B) Representative bacterial centerlines (red dots) for each wild-type strain fitted with a generalized sine function, (black dashed line). (C-E) Probability density for cell length (µm) (C), helical radius (µm) (D), and helical pitch (µm) (E) of LSH100 (n=262), B128 (n=272), and PMSS1 (n=215) bacteria. (F) Percent of the total bacterial population with a given flagellum number (n=104-110 bacteria). [Martínez et al., 2016] . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 38
3.3 A representative bacterial trajectory depicting the swimming motion of *H. pylori*. (A) A representative bacterial trajectory of *H. pylori* is shown segmented into forward (blue) and reverse swimming directions (red). In our analysis, the direction of swimming observed at the start of the video was considered forward. A reversal in swimming direction (green) was identified when bacteria exhibited a large angle change ($\Delta \theta > 110$ degrees). Upon a reversal, bacteria were assumed to continue swimming in the reversed direction (red) until another reversal took place. (B) Instantaneous forward and reversal swimming speeds and (C) change in swimming angle ($\Delta \theta$) of the bacterium in panel A over the course of time it was tracked (X (green) denotes reversals in swimming direction).

3.4 Probability density summarizing speed distributions for LSH100, B128, and PMSS1 bacteria swimming in broth (BB10) (A), in viscous solutions of PGM (15 mg mL$^{-1}$) (B), and in gel-like PGM solutions (30 mg mL$^{-1}$) (C).

3.5 Fractions of immobilized bacteria in viscous PGM media. The percentage of immobilized bacteria was calculated by dividing the total number of non-motile cells (bacteria that exhibit displacements $< 0.3 \mu$m) by the total bacterial population examined, as described in the Materials and Methods (3.2.5). [Martínez et al., 2016]
3.6 Speed distributions for wild-type and Δcsd6 isogenic straight rod mutants in broth (BB10) and viscous PGM media (15 and 30 mg mL\(^{-1}\)) (A,B), and in broth (BB10) and viscous MC media (10 and 15 mg mL\(^{-1}\)) (C,D). One of two or three independent experiments are shown. (E,F). Distribution of speed standard deviations, \(σ_{bac}\), for wild-type LSH100 (E) and PMSS1 bacteria (F) compared to their respective isogenic straight rod mutants in viscous solutions of PGM at 15 mg mL\(^{-1}\). The distributions show similar speed variation profiles between wild-type and straight rod mutants, where K-S p = 0.31 for LSH100 vs. LSH100 Δcsd6, and p = 0.51 for PMSS1 vs. PMSS1 Δcsd6. [Martínez et al., 2016]  

3.7 Reversal frequency and ratio of forward to reverse speed for wild-type strains and shape mutants. (A, B) Dot plots summarizing the number of reversals per second of wild-type \(H. pylori\) strains and isogenic Δcsd6 straight rod mutants in LSH100 (A) and PMSS1 (B) strains in broth (BB10) and in viscous PGM or MC media. Mean values of reversals per second are shown as bolded black lines and error bars indicate one standard deviation from the mean. Data shown is from one of two or three representative experiments for each strain. (C) Dot plots summarizing individual ratios of median forward swimming speed to median reversal swimming speed acquired for each bacterial cell that reversed and maintained at least 3 instantaneous forward or reversal speed values while swimming in broth or viscous PGM solutions (15 mg mL\(^{-1}\)). Ratios are plotted on a log\(_2\) scale and median values are shown as bolded black lines. *K-S p < 0.05 was considered significant and ns = no significant difference. [Martínez et al., 2016]
3.8 *H. pylori* % track linearity in viscous PGM media, irrespective of cell shape.

Bar histograms show cell path trajectory as percent track linearity (%TL) between wild-type *H. pylori* strains (solid bars, A-C) and isogenic Δcsd6 straight rod mutants (hashed bars, B-C) in LSH100 and PMSS1 strains, as calculated from the ratio of straight-line velocity to curvilinear velocity in broth (BB10) and in viscous PGM or MC media. Error bars represent SD. [Martínez et al., 2016] 51

3.9 (A), Percent of the total bacteria, within in a given strain, with a given flagellum number of flagellum (n=83-105). (B), Speed distributions and (C), distribution of bacterias standard deviations in swimming speed, $\sigma_{bad}$, for B128 along with flagella mutants, fliO$_{ΔC}$ and sRNA$_{T}$, in PGM (15 mg mL$^{-1}$). [Martínez et al., 2016] 52

3.10 Reversal frequency, ratio of forward to backwards swimming speeds, and % track linearity for *H. pylori* and flagella mutants of *H. pylori*. (A) Dot plots summarizing the number of reversals per second acquired for wild-type B128 and isogenic flagella mutants in viscous PGM solutions (15 mg mL$^{-1}$). Mean values of reversals per second are shown as bolded black lines and error bars indicate one standard deviation from the mean. The fliO$_{ΔC}$ shows a significant difference to wild-type B128 (KS, p = 0.0114). (B) Dot plots summarizing individual ratios of median forward swimming speed to median reversal swimming speed acquired for each bacterial cell swimming in PGM (15 mg mL$^{-1}$). Ratios are plotted on a log$_{2}$ scale and median values are shown as bolded black lines. (C) Bar histograms showing % track linearity (%TL) for wild-type B128 and isogenic flagella mutants in PGM (15 mg mL$^{-1}$). Error bars show one standard deviation from the mean and mean %TL values are summarized in Table 3.3. *K-S p < 0.05 was considered significant and ns = no significant difference. [Martínez et al., 2016] 54
4.1 Rendering of a helix with pitch ($P$) and helical radius ($R$) and the calculated shape factor ($S_h$) for a bacterium with helical cell body. In each plot, the dependence of $S_h$ is shown for a single cell body parameter $L$ (A), $R$ (B), or $P$ (C) while keeping the other two parameters constant. The parameters that were not varied were maintained at a cell length $L = 3.1 \, \mu m$, helical pitch $P = 2.5 \, \mu m$, helical radius $R = 0.15 \, \mu m$, cell diameter $D = 0.56 \, \mu m$, flagellum length $l = 4.1 \, \mu m$, flagella pitch $p = 1.58 \, \mu m$, flagella helical radius $r = 0.14 \, \mu m$, and flagella bundle thickness $d = 0.07 \, \mu m$. The blue shaded regions represent the range (min to max) of the cell shape parameters observed experimentally for all wild-type strains. [Martínez et al., 2016] . . . 64

4.2 Calculated shape factor ($S_e$) for a bacterium with ellipsoidal cell body. In each plot, the dependence of $S_e$ is shown for a single cell body parameter $L$ or $D$ while keeping the other parameter constant. The parameters that were not varied were maintained at a cell length $L = 3.1 \, \mu m$, cell diameter $D = 0.57 \, \mu m$, flagellum length $l = 4.1 \, \mu m$, flagella pitch $p = 1.58 \, \mu m$, flagella helical radius $r = 0.14 \, \mu m$, and flagella bundle thickness $d = 0.07 \, \mu m$. The blue shaded regions represent the range (min to max) of the cell shape parameters observed experimentally for all straight rod mutants. . . . . . . . . . . . . . . 67

4.3 Smoothed histogram of measured swimming speeds (solid lines) and swimming speeds predicted by RFT (dashed lines) for LSH100 and LSH100 $\Delta$csd6. RFT swimming speeds were calculated for hundreds of bacteria (LSH100 n=262, LSH100 $\Delta$ csd6 n=481) using measured cell shape and flagella parameters (Figure 3.2 and data from [Sycuro et al., 2013]). For each strains the flagella torque was taken to be the estimated value in (Table 4.2). . . . 69
5.1 Trajectory of *H. Pylori* swimming in broth media along with plots of speed
(*v*) and absolute angle change \(|\Delta \phi|\) as functions of time. Red circles indicate
times identified to be reorientation events using our trajectory analysis
procedure. ................................................................. 76

5.2 Histograms of \(v_{\text{run}}, \theta_{\text{re}},\) and \(t_{\text{run}}\) for *H. pylori* swimming in broth media
(BB10). The black line indicates a gamma function fit to the \(t_{\text{run}}\) distribution
with shape parameter, \(a = 2.0 \pm 0.2,\) and rate parameter, \(\lambda = 1.5 \pm 0.2\). . . 77

5.3 Scatter plot of \(i\)th reorientation angle, \(\theta_i,\) versus the \(i + 1\)th reorientation
angle, \(\theta_{i+1},\) (A). Scatter plot of \(i\)th run speed, \(v_i,\) versus the \(i + 1\)th run speed,
\(v_{i+1},\) (B). Histogram of the relative speed difference \(\delta v\) (C). \(\delta v\) histogram is
fit to three Gaussians peaks with a best fit obtained for peaks located at at
\(\delta v = 0.05 \pm 0.02,\) \(\delta v = 0.51 \pm 0.01,\) and \(\delta v = -0.49 \pm 0.02.\) Data presented
for *H. pylori* swimming in BB10. ........................................ 78

5.4 Histograms of \(v_{\text{run}}, \theta_{\text{re}},\) and \(t_{\text{run}}\) for *H. pylori* swimming in BB10 (blue),
PGM 15 mg mL\(^{-1}\) (red), and PGM 30 mg mL\(^{-1}\) (green). Black lines indicate
a gamma function fit to the \(t_{\text{run}}\) distribution with fit parameters summarized
in Table 5.1................................................................. 80

5.5 Histograms of \(v_{\text{run}}, \theta_{\text{re}},\) and \(t_{\text{run}}\) for *H. pylori* swimming in BB10, MC 10
mg mL\(^{-1}\), and MC 15 mg mL\(^{-1}\). ........................................ 82

5.6 The mean squared displacement, velocity autocorrelation function, and direc-
tional autocorrelation function for all bacteria in BB10, PGM 15 mg mL\(^{-1}\) (PGM15),
and PGM 30 mg mL\(^{-1}\) (PGM30) segmented into populations of bacteria which
were observed to reverse and those which were not observed to reverse. The
green and blue line indicate comparisons to theoretical random walk de-
scribed in the text. ......................................................... 85
6.1 Mean squared displacements calculated every thirty seconds for 3.0 µm beads in 5% gelatin solutions which have been cooled from 35° C to 26° C, 25° C, or 24° C. .......................................................... 92

6.2 The storage ($G'$) and loss ($G''$) moduli calculated from the mean squared displacement data for a 5% gelatin solution cooled from 35° C to 24° C. Dashed lines indicate power law fit to the 8.5 minute data. Power law exponents for $G'$ and $G''$ were within error with the exponent $n = 0.73 \pm 0.07$. ....... 93

6.3 Mean squared displacement for 3.0 µm beads in 1.5% and 3.0% after 10, 30 and 50 minutes at 25° C. .......................................................... 94

6.4 *H. pylori* swimming in a 1.5% gel. Individual bacteria trajectories are overlaid on the image in different colors. .................................................. 95

6.5 Number of motile bacteria as a function of gelatin concentration. Data from videos obtained up to 30 minutes after bacteria was added to each gelatin solution. .......................................................... 96

6.6 (A) Single image of a bacterium which rotated its cell body but did not translate (white line indicates the intensity profile line used to estimate the cells center). (B) The plot of the intensity profile along the profile line, the bacteria location is estimated as being the location of minima in the intensity. Trace of the minima in the intensity profile as a function of time. (C) The oscillating position of the cells center is a result of cell rotation and gives an estimated rotation rate of $\omega_b = 1 \pm 0.1$ Hz. .................................................. 97

6.7 Average swimming speed plotted as a function of gelatin concentrations. Error bars indicate standard error of the mean. .................................................. 98

6.8 Probability distribution of reorientation angles, $\theta_{re}$, in gelatin solutions at concentrations of 1.5%, 2.0%, 2.5%, and 3.0%. .................................................. 99
Chapter 1

Introduction

1.1 Motivation and Background

Motility is a key factor in the ability of bacteria to properly thrive in their niche environment. Bacteria have evolved several motility mechanisms designed to overcome the unique challenges present in their microenvironments. Study of these motility mechanisms has led to a better understanding of bacteria microbiology, along with application in engineering and the physical sciences. In this work, I examine the motility of the bacterium *Helicobacter Pylori*, and address the questions of what swimming behavior the bacteria uses and how its unique helical cell shape affects it swimming.

Motility is required for *H. pylori* to successfully colonize the human stomach [Lertseth-takarn et al., 2011], however little is known about the swimming strategy the bacteria uses. Previous research has shown that mutated strains of *H. pylori* lacking motility or the ability to sense attractants have reduced host colonization [Lertsethtakarn et al., 2011]. However, little research has been dedicated to characterize *H. pylori*’s swimming strategy and how it affects colonizing the mucus layer.

In addition to swimming behavior, a long-held dogma is that *H. pylori*’s helical cell shape gives it an advantage in penetrating the mucus layer covering the stomach epithelium. When bacteria swim their cell bodies rotates which, in combination with their helical geometry, results in a screw like cell motion that is thought to provide additional propulsion through
the mucus layer. To date, there have been few tests of this hypothesis and little is known about how helical cell shape affects motility. In general, what role cell morphology plays in bacterial swimming is an open question.

In this thesis, I characterize *H. pylori*’s swimming behavior and the role its helical cell shape plays in swimming. We show that these bacteria are found to use predominately a run-reverse swimming strategy, and alter their swimming behavior in mucin solutions showing decreased reversals. Experimental and modeling results show that *H. pylori*’s helical cell shape results in slightly faster swimming speeds when compared to rod shape mutant. However, the increase in swimming speeds is small compared to other factors that affect motility. In addition, I examine how *H. pylori*’s motility is altered in a polymer environment, including pig gastric mucin solutions analogous to mucus layer of the stomach. In the following sections of this chapter the background for my research is provided in greater detail.

1.2 *Helicobacter Pylori*

*Helicobacter Pylori* (*H. pylori*) is a gram-negative multi-flagellated bacterium with a helical cell shape, for which its name is derived [Marshall, 2002]. Figure 1.1 shows a TEM image of a single *Helicobacter Pylori* cell. Cells vary in shape with approximate cell lengths of \( \sim 3-5 \) \( \mu \text{m} \), cell thickness \( \sim 0.5 \) \( \mu \text{m} \), helical radius \( \sim 0-0.25 \) \( \mu \text{m} \), and helical pitch \( \sim 2-4 \) \( \mu \text{m} \). Bacteria have sheathed flagella which all extend from the same spot on the cell body with flagella numbering from one to seven per cell.

Physiologically *H. pylori* is the primary cause of gastric and peptic ulcer disease; with pathogenic links also made to gastric cancer [Peek and Crabtree, 2006]. It infects approximately 50% of the world’s population and is able to persist and cause chronic infection for long periods of time [Salama et al., 2004]. The bacterium’s pathogenic links and ability to colonize the human stomach, an environment long thought to be too harsh for bacteria to survive in, made its discovery a great achievement.
1.2.1 Discovery and Health Implications

*Helicobacter Pylori* was discovered and linked to human stomach ulcers by Barry Marshall. In now famous experiments, Barry Marshall and Robin Warren accidentally left petri dishes with bacteria from gastritis patients incubating over Easter holiday in 1983, only to return five days later and find small colonies growing [Marshall et al., 1985b]. This accident was the first time the bacteria was experimentally cultured. There was initially doubt from the scientific community as to whether this new bacteria was the cause of gastritis or simply a byproduct. In later experiments, Marshall inoculated himself with cultures of the bacteria and subsequently formed chronic gastritis 3 days later [Marshall et al., 1985a]. For the discovery of *Helicobacter Pylori* and its link to gastritis Marshal and Warren were awarded the 2005 Nobel Prize in Physiology and Medicine.

The exact means of human infection have yet to be fully understood. The bacterium is more prominent in undeveloped countries or in poor hygienic conditions and infection is often acquired by children. Interestingly, countries which have gone from undeveloped to developed have seen a marked decreased in *H. pylori* infection. Transmission can occur by a
1.2.2 Colonization of the Human Stomach

Once an individual has been infected, the bacterium seeks to colonize in the mucus lining of the human stomach. Figure 1.2 shows a simple diagram of *H. pylori* infection in the stomach [Y. tambe, ]. Bacteria first use their motility to swim from the gastric fluid into the mucus layer covering the epithelium. To survive in the harsh acidic stomach lumen, pH 2, and persist in the mucosal layer covering the stomach epithelium, *H. pylori* produces large quantities of the Ni2+ containing enzyme urease. Urease production is approximately 6% of the total protein production of *H. pylori* and studies have found cells lacking the ability to produce urease show decreased stomach colonization [Eaton et al., 1996]. Urease
hydrolyzes urea to produce ammonia, \( \text{NH}_3 \), and carbon dioxide, \( \text{CO}_2 \), as displayed in figure 1.2. Since ammonia is slightly basic this chemical reaction provides a means for \( H. \text{ pylori} \) to regulate its internal and external pH [Montecucco and Rappuoli, 2001]. Based on this the generally accepted view is that \( H. \text{ pylori} \) uses urease to raise the pH of its surroundings to a biologically acceptable level and uses its motility to swim through the mucus lining to the gastric epithelium. Once in the mucus, bacteria adhere to epithelium cells and cause mucosal damage, inflammation, and mucosal cell death.

Recent work by Celli et al. also relates the elevation of pH to enabling the motility of the bacterium across the mucus layer. Rheology experiments with pig gastric mucin (PGM), a homolog to human mucin, showed that raising the pH from 4 to 7 caused PGM gels to degel and form a solution [Celli et al., 2005, Celli et al., 2007, Celli et al., 2009]. Using light microscopy and two-photon fluorescence Celli et al. showed that, in the presence of urea, \( H. \text{ pylori} \) raises the pH and induces a gel-sol transition in PGM causing bacteria to transition from being immobile in a PGM gel at pH 2 to mobile in a PGM solution at pH 7. This suggests that \( H. \text{ pylori} \) produces urease to increase the pH so as to both increase its survivability in the stomach lumen and enable penetration into mucus layer by de-gelling it.

1.2.3 Motility and Morphology

While the requirement of motility for colonizing is well understood, details of swimming behavior and the role that cell shape plays in motility have yet to be fully characterized. Chemotaxis experiments with \( H. \text{ pylori} \) have shown that bacteria sense gradients of pH, bicarbonate, and urease [Lertsethtakarn et al., 2011]. \( H. \text{ pylori} \) has been observed to under-go stops, reversals and when placed in a pH gradient bacteria were found to increase the frequency of reversals so as to move away from the region of low pH [Howitt et al., 2011]. This behavior is caused by the flagella motor protein, ChePep [Howitt et al., 2011]. Several other flagella protiens have been identified and mutants strains lacking these proteins can also have altered swimming behavior [Lowenthal et al., 2009]. To date, detailed characterization
of *H. pylori*’s swimming behavior has not been done. In addition, *H. pylori*’s swimming behavior in a mucin environment has yet to be explored.

Cell morphology can also affect swimming speed and a bacterium’s ability to sense chemotactic gradients [Dusenbery, 1998, Young, 2007], and *H. pylori*’s helical cell shape has long been thought to give it a motility advantage. The idea that a helical cell shape provides a swimming advantage was originally proposed by Berg and Turner, who suggested that a helical cell shape would result in additional corkscrew-like propulsion for a bacterium moving in viscous environments [Berg and Turner, 1979]. Testing their prediction Ferrero and Lee observed that in highly viscous methylcellulose solutions of varying viscosity (>100 mPa), different clinical strains of the helical-shaped bacteria *Campylobacter jejuni* were more motile than rod-shaped bacteria of *Vibrio cholerae, Salmonella enteritidis*, and *Escherichia coli* [Ferrero and Lee, 1988]. Later experiments by Karim et al. showed that *H. pylori* and *C. jejuni*, both helical shaped bacteria, swam faster in liquid broth than *E. coli* [Karim et al., 1998]. However, these studies compare different species of bacteria, which can have differences in flagella morphology, number and arrangement, and motor output, making conclusions on the influence of cell body shape on motility in viscous solutions difficult to interpret. Ideally, experiments comparing bacteria with only shape alteration in several different polymer solutions would best test what role cell shape and solution properties has on swimming.

Recently genetic screens have been used to identify cell shape-determining (*csd*) genes required for *H. pylori*’s characteristic helical cell morphology [Sycuro et al., 2010, Bonis et al., 2010, Sycuro et al., 2012, Sycuro et al., 2013]. Several *csd* genes encode peptidases that modify the bacterial cell wall, composed of peptidoglycan, which is responsible for rigidity and cell shape in most bacteria [Cabeen and Jacobs-Wagner, 2005]. Elimination of the peptidoglycan peptidases Csd4 or Csd6 yields bacteria with straight rod morphology [Sycuro et al., 2012, Sycuro et al., 2013]. Experiments with wild-type *H. pylori* and cell shape mutants with a straight rod morphology show impaired stomach colonization, suggesting helical cell shape is important for initial colonization and/or persistence in the
stomach [Sycuro et al., 2010, Bonis et al., 2010, Sycuro et al., 2012]. Straight rod cell shape mutants also have decreased motility in semi-solid agar motility tests when compared to their parent wild-type strain [Sycuro et al., 2012]. These results indicate that the helical shape of \textit{H. pylori} is important for colonization and may play a role in bacteria swimming.

\textit{H. pylori} has the added challenge of having to swim in mucus polymer environment. The study of bacterial motility in polymer solutions has proven difficult due to the complex effects of shear-thinning and viscoelasticity. In addition, there isn’t a complete hydrodynamic framework for modeling bacteria motility in polymer solutions. Despite this, several important experimental and theoretical results have provided a groundwork for examining these problems. Several studies have shown that as the viscosity of the polymer solution increases bacteria increase their swimming speed to a peak followed by a continued decrease in swimming speeds [Ferrero and Lee, 1988, Shoesmith, 1960, Schneider and Doetsch, 1974]. Berg and Turner were the first to explain this behavior by suggesting that the cell body and flagella experience different drag [Berg and Turner, 1979]. They suggested that the cell body experiencing drag resulting from the polymer network while the size of the flagella results in it only experiencing the drag from the fluid environment [Berg and Turner, 1979]. This model was later mathematically modeled by Magariyama and Kudo and found to fit experimental results [Magariyama and Kudo, 2002]. An alternative model where the bacterium’s cell body and flagella experience different viscosities because of shear thinning effects has also been developed [Martinez et al., 2014]. In this model, the flagellum of a bacteria experiences a different viscosity because its rapid rotation ($\omega = 100 - 1000$ Hz) causes shearing thinning resulting in a lower effective viscosity.

### 1.3 Bacterial Swimming

Bacterial swimming is generally discussed in two regimes: hydrodynamics and statistical mechanics. When interested in hydrodynamics and how cell and fluid parameters alter swimming it is informative to examine the simplified cases, such as a single bacterium swimming at a constant speed. The hydrodynamic equations governing these cases can be
written and under certain assumptions solved, usually through the use of computational
techniques. These simplified cases allow one to explore cell motion and fluid properties
affect the swimming speed, and forces and torques on the cell.

Alternatively to this, when interested in the time variability of bacterium swimming and
how bacteria navigate their environment, it is common to examine the chemotactic behavior
of bacteria and the statistical properties of the position and speed of bacteria. Bacteria swim
in runs during which their speed and swimming direction are relatively constant. These
runs are interrupted by reorientation events during which bacteria change their speed and
swimming direction. This type of swimming behavior results in a stochastic-like swimming
trajectory, which can be described using random walk statistical models.

1.3.1 The Hydrodynamics of Bacterial Swimming

The hydrodynamics of swimming bacteria are much different than the macroscopic hydro-
dynamics we observe every day. To understand why, consider the Navier-Stokes equation for
an incompressible fluid with flow field $\mathbf{u}$, density $\rho$, viscosity $\eta$, and surrounding pressure, $P$.

$$\rho \left( \frac{\partial}{\partial t} + \mathbf{u} \cdot \nabla \right) \mathbf{u} = -\nabla P + \eta \nabla^2 \mathbf{u} \tag{1.1}$$

$$\nabla \cdot \mathbf{u} = 0 \tag{1.2}$$

The left side of the Navier-Stokes equation (1.1) is made up of inertial terms representing
the acceleration of the fluid. The right side is made of a pressure gradient and viscous drag
term. Equation 1.2 states that the fluid is incompressible.

In the case of steady flow around an object with speed $U$ and a characteristic size $L$
the Reynolds Number, $Re$, is defined as the ratio of the typical inertial term, $\sim \rho U^2/L$, to
the typical viscous term, $\sim \eta U/L^2$, $Re = \rho U L/\eta$. A human swimming in a pool ($L \sim 1$
m, $U \sim 1 \text{ m/sec}$, $\rho \sim 10^{-3} \text{ kg m}^3$, $\eta \sim 10^{-3} \text{ Pa s}$) has a $Re$ of $\sim 10^3$ meaning the effects of
inertial are much larger than viscous effects. In stark contrast to this, a bacteria swimming
in water ($L \sim 10^{-6} \text{ m}$, $U \sim 10^{-6} \text{ m/s}$, $\rho \sim 10^3 \text{ kg m}^3$, $\eta \sim 10^{-3} \text{ Pa sec}$) has a $Re$ of $\sim$
10^{-6}, indicating viscous effects are significantly larger than inertial effects. Objects moving at low Reynolds number cannot rely on inertia as a means to move any meaningful distance and are considered to be moving in a viscous dominated regime. For example, a bacterium swimming at \( \sim 20 \, \mu\text{m}/\text{sec} \) in water will coast due to its inertial for \( \sim 1 \, \mu\text{m} \), a distance small compared to the diameter of an atom [Berg, 1993].

For low \( Re \) equation 1.1 can be simplified to the Stokes equation (1.3). The Stokes equation has two properties that are important for bacteria swimming: time invariance and linearity.

\[
-\nabla P + \eta \nabla^2 \mathbf{u} = 0
\]

(1.3)

The time invariance of the Stokes equation dictates that any periodic swimming mechanisms will result in a net zero displacement. This is Purcell’s famous Scallop Theorem, which states that to produce a thrust at low \( Re \) a swimmer must deform in a way that is not invariant under time reversal [Purcell, 1977]. To meet this requirement bacteria have evolved several novel swimming mechanisms to produce an asymmetric drag, such as the rotation of helical flagella.

The linearity of the Stokes equation results in the kinematics of a swimming bacterium being linearly related. Specifically, the forces, \( F \), and torques, \( N \), on a bacteria can be said to be linearly related to the speed, \( U \), and rotation rate \( \omega \) of the bacteria. Mathematically this can be written

\[
\begin{bmatrix}
F \\
N
\end{bmatrix} =
\begin{bmatrix}
M_1 & M_2 \\
M_3 & M_4
\end{bmatrix}
\begin{bmatrix}
U \\
\omega
\end{bmatrix}
\]

(1.4)

here \( M_1, M_2, M_3, \) and \( M_4 \) are called resistive coefficients. This matrix can be further simplified through the use of the Purcells Reciprocal Theorem, which states that the resistive matrix must to be symmetric, \( M_3 = M_2^T \). In addition, depending on what symmetries the bacterium may have the resistive matrix maybe further simplified. Solving for the resistive coefficients can be challenging as they depend on the geometry of the bacterium, however, several theoretical and computational tools have been developed [Lauga and Powers, 2009].

The linearity of the Stokes equation also allows for the tools of superposition to be used
to solve for the flow field. The Greens function solution to the Stokes equation given a
forcing term $\delta(r - r')F$ is

$$u(r) = G(r - r')F$$

$$G(r) = \frac{1}{8\pi\eta} \left( \frac{I}{|r|} + \frac{rr}{|r|^3} \right)$$

here $G(r)$ is the Oseen tensor and $G(r - r')F$ is called a Stokeslet [Lauga and Powers, 2009]. Stokeslets describe the resultant flow field produced by a force point, and thus can be thought of as the hydrodynamic equivalent of a point charge. The problem of solving for the flow field for a swimming bacterium can then be thought of as finding the correct arrangement of Stokeslets in order to match the boundary conditions. For a swimming bacterium the surface of a bacterium and its flagella are usually assumed to be a no-slip boundary condition and in the bacterium frame of reference, its surface can then be considered a time varying boundary condition for the surrounding fluid. The complex shape and dynamics of the bacterial surface usually prevent the flow field from being solved analytically in this way, however, several approximation techniques have provided insights.

Two common techniques that have proven useful in better understanding the hydrodynamics of swimming of bacteria are resistive force theory (RFT) and slender body theory (SBT). Resistive force theory (RFT), also called local drag theory, attempts to calculate the resistive coefficients by assuming that the flow field is approximately local. For a flagellated bacterium, RFT assumes the flagella is a rod composed of segments, each of which does not interact with each other hydrodynamically. Using the linearity of the Stokes equation, the total flow field can be obtained by summing the flow fields from each individual segment. Taking the limit as the number of segments goes to infinity this sum becomes an integral over the length of the flagellum. The fluid field can then be related to the force and torque on the flagella. This approach is relatively simple and can provide some general insights, however, the method fails when the object being considered is not sufficiently slender. Slender body theory uses a similar approach but instead of assuming the field is local and ignoring long-range interactions it assumes objects are sufficiently slender. In the case of bacteria flagella, SBT can be used to calculate the forces and torques on the flagella.
under the assumption that the flagella thickness is small compared to its length. While SBT does result in more accurate calculations the limitation that the object is sufficiently slender makes it inapplicable for many scenarios.

Recently a regularized Stokelets method (RSM) has been developed and used with success to more accurately model rotating helices and swimming bacteria [Cortez et al., 2005]. Compared to RFT and SBT, RSM has been found to more accurately model the flagella and is more suited for modeling objects with thick or unusual geometries [Rodenborn et al., 2013]. It is significantly more computationally expensive than RFT and SBT, but can still be done on a desktop computer.

1.3.2 Bacterial Swimming Strategies and Chemotaxis

Classically the swimming behavior of bacteria has been described using the run-tumble model first developed to characterize the swimming of *E. Coli* [Berg et al., 1972]. In this model, bacteria swim in straight trajectories called runs which are interrupted by events called tumbles (Figure 1.3A). During a tumble bacteria do no actively swim but undergo translation and rotational diffusion that causes the bacteria’s swimming direction to randomly change from one run to the next. This change in swimming direction from run to run allows the bacteria to gradually explore its environment in a random walk fashion. Further research by Berg and others showed that chemo-attractants cause *E. Coli* to vary their tumble rate allowing them to bias their random walk to swim towards nutrient-rich regions [Berg and Tedesco, 1975]. Other bacteria have been observed to bias their motion in a similar matter, but several bacteria have been observed to use alternative swimming behavior to move throughout their environment. Two alternative swimming strategies recently characterized are run-reverse and run-reverse-flick.

Bacteria having run-reverse swimming show similar to trajectories to run-tumble, however instead of a tumble event the change in swimming direction is biased towards a reversals (angle changes by \(\approx 180^\circ\)) (Figure 1.3B). Several marine bacteria have been observed to use this behavior [Mitchell et al., 1996] and it is theorized to be more effective for remaining
Bacteria having a run-reverse-flick behavior have a similar run-reverse behavior but undergo a rapid change in swimming direction termed a flick after their reversal (Figure 1.3C) [Xie et al., 2011]. This flick is caused by a buckling instability in the flagellar hook that results in a change in swimming direction of $\sim 90^\circ$ after reversals.

While the details of these swimming dynamics are different, they can both be modeled using random walk models. For run-tumble swimming, experiments by Berg found cells switch back and forward between runs and tumbles at random resulting in a random walk like trajectories [Berg, 1993]. The random occurrence of runs and tumbles implies they occur with a fixed probability per unit time, and the time between tumbles follows a Poisson distribution. This results in cells undergoing a three-dimensional random walk.

For run-reverse and run-reverse-flick similar, although more complicated, models have been proposed [Taktikos et al., 2013]. These models explore how altering the time between reorientation events, angle change after events, and swimming speed affects a bacterium’s ability to explore its environment.
Chapter 2

Materials and Methods

This chapter provides an overview of the experimental methods used in this work and the scientific principles of these methods. Further details can be found in the chapters where each methods measurements are discussed.

2.1 Bacteria Strains and Culturing

Several wild-type strains of *H. pylori* were used in this study. The term wild-type refers to bacteria that have the form that usually occurs in nature, however, several wild-type strains can exist, each differing slightly in their genetic makeup. These genetic differences can result in observable differences between wild-type strains. In this work, wild-type strains were used that differed in cell shape, flagella shape, and the numbers of flagella they produced. By comparing the motility of these strains we were able to examine how their morphological differences affected their motility and swimming behavior. In addition to different wild-type strains, cell shape and flagella mutants of *H. pylori* were also used in this study.

*H. pylori*’s helical cell shape has been linked to the sacculus, a mesh of peptidoglycans cross linked by peptides that form outside the cell membrane. Work by Sycuro et al. [Sycuro et al., 2010] found that genetic mutants of *H. pylori* lacking cell shape determinant (*csd*) or curved cell morphology (*ccmA*) genes have altered cell shapes compared to their parent strains. They found these genes code for specific peptidases and proposed that *H. pylori*’s
helical cell shape is a result of these peptidases hydrolyzing the peptidoglycan cross links in the sacculus. Figure 2.1 shows a diagram on how this is thought to occur. Hydrolysis of cross links along a straight line along the sacculus results in curved, C-shaped cells, while hydrolysis along an angled line along the sacculus results in curved and twisted cell. In this study, we used cell shape mutants produced by our collaborators at the University of Washington that lack the csd6 gene. These mutants, termed Δcsd6, have decreased peptidase activity and a straight rod morphology [Sycuro et al., 2013].

In addition, to cell shape mutants we also used flagella mutants with varying number of flagella. Previous studies have found that modification of the flagella protein FliO results in decreased number of flagella [Tsang and Hoover, 2014]. In contrast, bacteria with an increased number of flagella are created using a small non-coding RNA mutant (s RNAT) [Sharma et al., 2010]. Use of these mutants allows for examination of how the number of flagella a bacteria has affects their swimming behavior. We also use a strain that lacks motility due to the deletion of motb gene.

To use these wild-type and mutant strains in microscopy experiments they must be cultured to an appropriate state. The culturing procedure for *H. pylori* is well developed and involves first growing bacteria on agar plates and later moving them to liquid culture media. Agar plates are composed of blood, usually from horses, and nutrients held in an
agar gel. Bacteria from frozen stock are streaked onto these agar plates using a sterile inoculating loop and placed in an incubator. The incubator maintains the temperature and gas mixture that promotes growth cell growth and colony formation. After two to four days bacteria colonies are readily visible. At this point, bacteria are moved from agar plates to a liquid growth media. This is done by taking a swab of a colony from the agar and mixing it into liquid growth media using a sterile inoculating loop. Liquid cultures are then placed back in the incubator for further growth. To promote motility and prevent cells from clumping together liquid cultures are constantly agitated using a shaker.

The concentration of bacteria in liquid samples is monitored by measuring the absorbance using a spectrophotometer. Initially, bacterial growth is slow as bacteria adapt to the environment and start to produce molecules important for cell growth, this phase is termed the lag phase. Once bacteria have adapted and the necessary molecules for cell growth become available bacteria grow exponentially, this phase is termed the exponential phase. Exponential growth continues unless the concentration of nutrients become depleted and the bacterial population becomes constant, this phase is termed the stationary phase. As the bacteria concentration increases during the exponential phase the liquid culture becomes increasingly turbid and absorbance increases. By measuring absorbance at different times during the exponential phase, growth curves and the doubling time (the time it takes for the population to double while in the exponential phase) were obtained. Previous studies of \textit{H. pylori} have found bacteria are most motile during the mid to late exponential phase when the absorbance of the liquid culture lies in the range of 0.5-0.7 [Worku et al., 1999]. Once liquid cultures reach this range of absorbance, a sample of liquid culture is taken and either diluted with liquid growth media or added to a polymer solution. These bacteria solutions are then imaged using a phase contrast microscope.

\section{2.2 Microscopy and Particle Tracking}

To measure a bacterium’s swimming behavior one needs to obtain the position of the bacterium as a function of time. This can be done using several experimental methods with
the most common being the use of video microscopy and particle tracking algorithms. In this section, I outline the microscopy and particle tracking techniques used in this work.

2.2.1 Phase Contrast Microscopy

Modern microscopy techniques make it possible to image bacteria at several scales with varying positional and temporal resolution. Popular imaging modalities for observing multiple bacteria include phase contrast microscopy, differential interference contrast microscopy (DIC), and fluorescence microscopy. For the purposes of studying motility, phase contrast and DIC provide the ability to resolve multiple cells and, with the use of a CCD or CMOS camera, capture images at a suitable rate for tracking bacteria frame by frame. In this work, we focus on the use of phase contrast microscopy.

In phase contrast microscopy an image is produced by transforming differences in the phase of light into differences in amplitude [Murphy, 2002]. The light coming from a microscope sample can be thought of as being made of light that interacted with the specimens in the sample and light that did not interact, background light. Light that interacted with the specimens in the sample will be phase shifted by \( \delta = \frac{2\pi(n_2 - n_1)t}{\lambda} \) where \( \lambda \) is the wavelength of incident light, \( t \) is the thickness of a specimen and \( n_1 \) and \( n_2 \) are the refractive indices of the surrounding media and specimen, respectively. Specimens in a sample that have refractive indices differing from the surrounding media will produce phase shifted light.

From a microbiology standpoint, the collection of molecules within a bacterium results in a difference in refractive index between the bacterium and the surrounding media, and thus light from a bacterium is phase-shifted light relative to the background light.

The human eye perceives light intensity not phase, to observe the phase shift described above two elements are added to the light path of a standard microscope: a condenser annulus and a phase plate [Murphy, 2002]. These elements cause the background light to be phase shifted and decreased in intensity. As a result, when the background light and phase shifted light from the specimen interfere at the image plane, they partially cancel one another producing an image whose intensity is predominately due to the phase shifted light.
2.2.2 Fluorescence Microscopy

In this work fluorescence microscopy is used to track fluorescent particles for microrheology experiments. In contrast to other imaging modalities which depend on measuring changes in the intensity or phase of light, fluorescence microscopy involves measuring emitted light produced by changes in the energy states of fluorescent molecules. The electrons of fluorescent molecules are put into an excited state when illuminated with light at a specific wavelength. After excitation electrons rapidly decay back to the ground state and emit fluorescent light. Because some energy is lost in this process, the emitted light has lower energy than the excitation light and thus has a longer wavelength. The specific wavelengths of excitation and emission light depend on the fluorescent molecule. A fluorescence microscope sends light through a bandpass filter so the sample is only illuminated with wavelengths corresponding to the excitation wavelengths of the fluorescent molecules in the sample. The light emitted by these fluorescent molecules is then filtered so only wavelengths in the emission band are observed. This use of filters produces fluorescence microscopy images were only the objects contained the fluorescent molecules are observed. In our experiments, we image
fluorescent beads that are saturated with fluorescent molecules in various solution. When viewed under a fluorescence microscope (Figure 2.3) beads appear as bright objects on a black background. The high contrast images produced by fluorescence microscopy makes tracking beads using particle tracking algorithms more accurate than images obtained using other microscopy techniques such as bright field or phase contrast.

2.2.3 Particle Tracking

In this section, an overview of how we locate the position of objects within an image is provided. Automated particle tracking software has made it possible to locating and tracking objects in an image with high precision. Two different software are used in this study: a commercially available tracking software called Volocity (v6.1) (Improvision, Perkin Elmer, 2011) which uses a centroid tracking method to find the center of intensity of an object and an open source MatLab code developed and written by Rogers et al. [Rogers et al., 2007]
which uses a local quartic fit to the intensity profile to locate an object’s center.

Each image in a video can be thought of as a matrix $I$ where the elements of $I$ are the intensities of each pixel in the image. Images of bacteria obtained using phase contrast microscopy show bacteria as dark objects on a light background while images of fluorescent beads obtained using fluorescence microscopy show beads as bright objects on a dark background. A first approximate for the locations $(x_0, y_0)$ of bright (beads) or dark (bacteria) objects can be obtained by locating the local minima or maxima of intensity in $I$. However, this method has several problems including inaccurate tracking of objects if they are illuminated unevenly and attributing multiple positions to the same object if it has multiple local minima or maxima. Tracking can be greatly improved by refining the position using a location algorithm. Two algorithms are used in this work the centroid method and fitting the objects local intensity profile.

The centroid method assumes a specific shape for the object and then locates the center of intensity of the object using that assumed shaped. For example, if the object is assumed to appear as a circle in the image with radius $w$ its centroid position $(x, y)$ is calculated by finding the center of its intensity according to equation 2.1 and shifting the initial position of the object using equation 2.2.

$$
\begin{align*}
\epsilon_x &= \frac{\sum_{i^2+j^2<w} i I(x_0 + i, y_0 + j)}{\sum_{i^2+j^2<w} I(x_0 + i, y_0 + j)} \\
\epsilon_y &= \frac{\sum_{i^2+j^2<w} j I(x_0 + i, y_0 + j)}{\sum_{i^2+j^2<w} I(x_0 + i, y_0 + j)} \\
(x, y) &= (x_0 + \epsilon_x, y_0 + \epsilon_y)
\end{align*}
$$

In our case, the initial position is taken to be the local minima or maxima $(x_0, y_0)$. To improve tracking accuracy the positional shifting can be done recursively until the correction to the position $(\epsilon_x, \epsilon_y)$ is below a specific threshold. This method has the benefit of allowing the user to try different shapes in order to improve their tracking accuracy. For example, if instead of a circle the object was assumed to appear as a box in the image with width $w$ the limits of equation (2.1) would be changed to $i + j < w$. The flexibility of this method makes it useful for tracking a variety of different objects, however, if objects have non-uniform
intensity the centroid method can result in a slight bias in the objects estimated position.

An alternative to the centroid method is to fit the local intensity profile of objects in the image. As with the centroid method, objects initial locations \((x_0, y_0)\) are obtained by finding local maxima and minima in the image. Using these initial locations one can fit the local intensity profile to an intensity function \(G(x, y)\). In the case of bright spherical beads, the local intensity profile can be fit well by a Gaussian function \(G(x, y) = Ae^{-(x^2+y^2)/B}\) \cite{Crocker1996}. A comparative study of the centroid method and fitting the local intensity to a Gaussian intensity function showed that a Gaussian fit results in decreased tracking bias and better performance when the signal to noise of the image is low \cite{Cheezum2001}.

For asymmetry objects, such as bacteria, it is beneficial to instead use an asymmetric function for \(G(x, y)\). Rogers et al. found that by taking \(G(x, y)\) to be a quartic polynomial \(G(x, y) = \sum_{i=0}^{i=4} \sum_{j=0}^{j=4} P_{ij}(x - x_0)^i(y - y_0)^j\) and using a weighted least-squared fitting of the intensity profile they could accurately track asymmetric objects \cite{Rogers2007}. Once a fit is computed the location of the maxima, or minima, of the quartic \((\epsilon_x, \epsilon_y)\) is used to shift the position of the object using equation (2.2). Similar to the centroid method, this shift can be done recursively to improve tracking accuracy.

Once the position of individual objects in each image is obtained the trajectories are constructed by linking those positions from frame to frame. Both tracking methods link objects from one frame to the next by minimizing the distance the objects travel from frame to frame. In addition, several parameters are used to prevent tracking objects that move out of the focal plane.

### 2.3 Trajectory Analysis

Using the tracking methods described above, we obtain hundreds individual trajectories of bacteria or beads. Each of these trajectories is analyzed to obtained important tracking parameters. For bacteria motility experiments, we obtain the parameters that characterize the swimming behavior of the bacteria while for microrheology experiments we calculate
the beads mean-squared displacement and use it to infer the rheological properties of the beads environment. Here I first describe how bacteria trajectories are analyzed to obtain the autocorrelation functions of the bacteria and segment their trajectories into runs and reorientations. I then describe the microrheology relationships that provide the basis for calculating the rheological properties of a material from the motion of particles in the material.

2.3.1 Bacteria Trajectory Analysis

Using the position vector of each bacteria \( \vec{r}(t) = x(t)\hat{e}_x + y(t)\hat{e}_y \) we calculate the velocity \( \vec{v}(t) \) and angle \( \phi(t) \) according to (2.3) and (2.4) where \( \tau \) is the time between frames.

\[
\vec{v}(t) = \frac{\vec{r}(t + \tau) - \vec{r}(t)}{\tau}
\]  

(2.3)

\[
\phi(t) = \arctan\left(\frac{v_y(t)}{v_x(t)}\right)
\]  

(2.4)

The position and velocity vectors for each bacteria are then used to calculate their mean squared displacement \( \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle \), velocity autocorrelation function \( \langle \vec{v}(t) \cdot \vec{v}(0) \rangle \), and directional correlation function \( \langle \dot{\vec{e}}(t) \cdot \dot{\vec{e}}(0) \rangle \) where \( \langle \ldots \rangle \) denotes a time averaging over the bacterium’s trajectory. These statistical measures provide insight into the time variability of a bacterium’s swimming behavior and allow for comparison to random walk swimming models. To further characterize the swimming behavior of the bacteria several additional swimming parameters are calculated.

Figure 2.4 shows a trajectory of a \emph{H. pylori} swimming in broth media (BB10), along with plots of its speed \( (v(t) = |\vec{v}(t)|) \) and absolute angle change \( (|\Delta\phi(t)| = |\phi(t + \tau)| - |\phi(t)|) \) as functions of time. The bacterium is observed to have periods during which swimming speeds are relatively constant and angle changes are small, termed runs. Runs are interrupted by periods of rapid speed and angle change, termed reorientations. To segment each trajectory into runs and reorientations I used a modified version of the method developed by Theves et al. [Theves et al., 2013] and Son et al. [Son et al., 2013]. The details of the method are described below.
Figure 2.4: Trajectory of *H. Pylori* swimming in broth media along with plots of speed \(v\) and absolute angle change \(|\Delta \phi|\) as functions of time. Red circles indicate times identified to be reorientation events.

Reorientation events were located by looking for large changes in \(|\Delta \phi(t)|\) and \(|\vec{v}(t)|\). First all local maxima in \(|\Delta \phi(t)|\) were located for each bacterium’s trajectory. If the angle change during a maxima, \(|\Delta \phi(t_{\text{max}})|\), was large compared to the rotational diffusion of the bacteria it was cataloged as a reorientation events (2.5). The criterion for how much large the maxima has to be relative to the rotational diffusion of the bacteria was set by a threshold variable \(\gamma\). We estimated the rotational diffusion constant of a bacteria by approximating it is an ellipsoid with semiminor axis \(a \approx 0.5\) and semimajor axis \(b \approx 3\), the rotational diffusion constant for this ellipsoid \(D \approx k_b T X (\ln(2b/a) - 0.5)/(8\pi\eta b^3/3) \approx 0.05\text{rads}^2/\text{sec}\) [Berg, 1993]. Using this rotational diffusion constant we found \(\gamma = 8.5\) to be sufficient to identify events during where bacteria actively reorientated their swimming direction.

\[
|\Delta \phi(t_{\text{max}})| > \gamma \sqrt{2D\tau} \tag{2.5}
\]
Once these reorientation events were identified the bacterium was assumed to stay in the reorientation state for a time $t_{re}$ calculated by examining the local angle changes $|Δφ(t_{max} + t_{re})|$. If $|Δφ(t_{max} + t_{re})|$ was large compared to the maxima $|Δφ(t_{max})|$ (2.6) we assumed the bacteria was still in a reorientation state. We used a threshold parameter $Γ$ to set how large angle changes must remain to be counted as part of the a reorientation event. We found $Γ = 0.7$ sufficient to identify events when a bacterium continues to have rapid changes in angle, while not misidentify runs as reorientation events.

$$|Δφ(t_{max})| - |Δφ(t_{max} + t)| > Γ|Δφ(t_{max})|$$

(2.6)

In addition to using angle changes to identify reorientation events, we also used changes in swimming speed to identify reorientations. For each bacterium we identified all minimum in the swimming speed, $v(t_{min})$. Reorientations were identified as events when the change in speed was found to be large in accordance with equation 2.7. The parameter $α$ sets how large the speed change must be to be counted as a reorientation. We found $α = 1.75$, sufficient to identify changes in swimming speeds corresponding to reorientations; this is equivalent to requiring that the relative speed change be greater than 175%.

$$Max(|v(t_{min} + τ) - v(t_{min})|, |v(t_{min} - τ) - v(t_{min})|) > αv(t_{min})$$

(2.7)

Bacteria identified to have these large speed changes were assumed to remain in the reorientation state using one of two criteria. Initially, we adopted the criteria used by Masson et al. [Masson et al., 2012] in which a bacterium is assumed to stay in the reorientation state as long as its speed is less than a cut off value, in our case we used half the average swimming speed of the bacterium $v(t) < \langle v(t) \rangle / 2$ where $\langle ... \rangle$ denote a time average over the trajectory. Results obtained using this criteria are shown in Chapter 3. In later analysis, we developed an alternative method in which after a large speed change the bacterium was assumed under go Brownian motion with an effective diffusion constant $D_{eff}$. If the bacterium’s displacement became larger than expected by Brownian motion the bacteria said not be in the reorientation state any longer. Mathematically the bacterium was defined to be in the reorientation state as long as equation 2.8 was valid. We found setting $D_{eff} = 0.01 \mu m s^{-1}$
allowed for identification of reorientation events and time during which bacteria became stuck or mobile. Analysis using this method is shown in Chapter 4.

\[ (\langle \vec{r}(t + t_{\text{min}}) - \vec{r}(t_{\text{min}}) \rangle)^2 < 2D_{\text{eff}}t \]  \quad (2.8)

Once reorientation events were identified using these methods each bacterium’s trajectory was segmented into runs and reorientations. Runs were characterized by the time the bacteria spent in the run \((t_{\text{run}})\), also called the run time, and the run speed, calculated by averaging the swimming speed over the run time \((v_{\text{run}} = \langle v(t) \rangle_{\text{run}})\). Reorientation events were characterized by the time spent in a reorientation \((t_{\text{re}})\) and by calculating the change in swimming direction that occurred as a result of the reorientation \((\theta_{\text{re}} = |\phi(t + t_{\text{re}}) - \phi(t)|)\).

### 2.3.2 Microrheology

Microrheology is the study of rheology at the microscale. Several experimental techniques exist for measuring the rheological properties of a material at the microscale, with particle tracking microrheology being one of the most popular. Particle tracking microrheology focuses on analyzing the trajectories of beads in the material in order to infer the rheological properties of that material. Using each bead’s position vector \((\vec{r}(t) = x(t)\hat{e}_x + y(t)\hat{e}_y)\) each of their mean squared displacements, \(\text{MSD} = \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle\), can be calculated. In a viscous fluid a bead undergoes Brownian motion with its MSD well described by equation 2.9.

\[ \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle = 2fD_b t \]  \quad (2.9)

Here \(f\) denotes the degrees of freedom of the bead and \(D_b\) is the diffusion constant of the bead. The diffusion constant for a sphere of radius, \(R\), in a low Reynolds number solution with viscosity, \(\eta\), and temperature, \(T\), is \(D_b = kT/6\pi\eta R\). Combining \(D_b\) with equation 2.9 one obtains the familiar Stokes-Einstein relation (2.10).

\[ \eta = \frac{f k T t}{3\pi R \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle} \]  \quad (2.10)

Using equation 2.10 makes it possible to calculate the viscosity of a fluid by measuring the MSD of beads in the solution, radius of the beads, and the temperature of the solution.
While this is an effective method for determining the viscosity of fluids, equation 2.10 breaks down for gels or viscoelastic materials. In these solutions beads are found to have sub-diffusive behavior with the MSD instead following a power law (2.11) [Cicuta and Donald, 2007].

\[
\langle (\vec{r}(t) - \vec{r}(0))^2 \rangle = At^\alpha, \alpha < 1
\] (2.11)

The value of \( \alpha \) provides a measure of how hindered the beads motion is and can be obtained from experimental bead trajectories by fitting their MSD. Using this form in the Stokes-Einstein equations results in equation 2.12.

\[
\eta = \frac{f k T t^{1-\alpha}}{3 \pi R A}
\] (2.12)

However, in actuality these materials have both viscous and elastic properties characterized by the materials storage (\( G' \)) and loss (\( G'' \)) moduli. Methods for calculating \( G' \) and \( G'' \) from bead mean squared displacements were first developed by Mason et al. [Mason et al., 1997] and later put on firm theoretical ground by Levine and Lubenskey [Levine and Lubensky, 2001]. They proposed a generalization of equation 2.10 to what is now called the Generalized Stokes-Einstein equation (2.13).

\[
G^*(\omega) = \frac{kT}{i\omega \pi R \langle (\vec{r}(\omega) - \vec{r}(0))^2 \rangle}
\] (2.13)

Here \( \langle (\vec{r}(\omega) - \vec{r}(0))^2 \rangle \) is the unilateral Fourier Transform of the MSD with frequency \( \omega \) and \( i = \sqrt{-1} \). Using this form of generalized Stokes-Einstein is difficult in practice due to the inaccuracies of numerically calculating the unilateral Fourier transform of experimental data with a finite range. Mason has developed an approximation to to generalized Stokes-Einstein equation (2.14) assuming the beads mean squared displacement can be approximated as power law in time.

\[
G^*(\omega) \approx \frac{kT}{\pi R \langle (\vec{r}(\omega) - \vec{r}(0))^2 \rangle \Gamma[1 + \alpha(\omega)]}
\] (2.14)

\[
\langle (\vec{r}(\omega) - \vec{r}(0))^2 \rangle = \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle |_{t=1/\omega}
\] (2.15)

\[
\alpha(\omega) = \frac{d \ln \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle}{d \ln t} |_{t=1/\omega}
\] (2.16)
Here $\Gamma$ denotes the gamma function.

$$\Gamma(x) = \int_0^\infty s^{x-1}e^{-s}ds$$ (2.17)

Using this approximation one can then calculate the storage $G'(\omega)$ and loss $G''(\omega)$ moduli as functions of time using equation 2.18 and 2.19 [Mason et al., 1997].

$$G'(\omega) = |G^*(\omega)| \cos\left(\frac{\pi\alpha(\omega)}{2}\right)$$ (2.18)

$$G''(\omega) = |G^*(\omega)| \sin\left(\frac{\pi\alpha(\omega)}{2}\right)$$ (2.19)

This makes it possible to measure the viscoelastic moduli of a material by analyzing the mean-squared displacement of beads in that material.
Chapter 3

Experiments on the role of Cell Morphology on Motility

3.1 Introduction

In this chapter, results obtained in collaboration with Professor Nina Salama’s group at the University of Washington and published in Molecular Microbiology [Martínez et al., 2016] are presented. In this work, we used morphology measurements of cell shape and flagellum parameters, and live-cell imaging of bacteria to examine their swimming behavior in several polymer solutions, including the physiologically relevant porcine gastric mucin (PGM). PGM is homologous to the human glycoprotein MUC5AC, the major secreted mucin expressed in the stomach mucosa by surface epithelial cells [Sellers et al., 1986, Bansil and Turner, 2006, Bansil et al., 2013]. PGM has been shown to exhibit similar rheological properties to human mucus scraped from the surface of the gastric mucosa, making it an ideal comparison to \( H. pylori \)'s niche environment [Schrager and Oates, 1974, Pearson et al., 1980, Bell et al., 1984, Bell et al., 1985]. Gastric mucus is believed to have two layers differing in mucin concentration, a thin firmly adherent, high concentration layer (around 30 mg mL\(^{-1}\)), and an overlying loosely adherent layer of mucus on the luminal surface (15 mg mL\(^{-1}\)) [Taylor et al., 2004]. Our experimental findings indicate that \( H. pylori \)'s cell body shape and flagellum number independently contribute to motility in viscous environments, including gastric mucin.
3.2 Materials and Methods

3.2.1 Bacteria Strains and Culturing

Three unrelated wild-type strains: LSH100, a derivative of the sequenced human clinical isolate G27 [Baltrus et al., 2009, Lowenthal et al., 2009], PMSS1, also called 10700 [Lee et al., 1997, Arnold et al., 2011], and B128 [McClain et al., 2009]; along with several mutant derivatives of these strains were used. To remove *H. Pylori*’s helical cell shape, generations of knockout isogenic mutants lacking helical cell morphology were produced in accordance with previous protocols [Sycuro et al., 2013]. Using similar protocols isogenic flagella mutants, motB (HPG27_772) was constructed in the LSH100 strain background and sRNA_T was generated in the *H. pylori* B128 background. These procedures allowed for experiments with wild-type bacteria, bacteria lacking helical cell shape, bacteria lacking motility, and bacteria with more or fewer flagella than their respective wild-type strain.

Bacteria were cultured on horse blood plates and in liquid media containing 90% (v/v) Brucella broth (BD Biosciences) and 10% fetal bovine serum (GIBCO) (BB10) in the absence of antimicrobials as previously described [Sycuro et al., 2010]. Cells were maintained at 37° C under microaerobic, low oxygen, conditions in a tri-gas incubator equilibrated to 10% CO₂ and 10% O₂ or using a BD GasPak pouch system (BD Biosciences). Bacteria were first cultured on horse blood plates which were incubated 24-72 hours before bacteria were moved to liquid cultures where they were incubated for another 12-16 hours under constant agitation before use.

3.2.2 Morphology Analysis

For each strain cell morphology analysis consisted of measuring a population of bacteria cell shape parameters, cell length, \( L \), helical pitch, \( P \), helical radius, \( R \), and cell thickness, \( D \), along with flagella parameters, flagella length, \( l \), and number of flagella, \( N \). To examine the cell shape each strain bacteria were grown in liquid culture to an optical density at 600 nm (O.D. (600)) of 0.3-0.7. Bacteria were fixed (4% Paraformaldehyde, 1X PBS,
and 25% Glycerol) and added to 0.1% poly-L-lysine (Sigma-Aldrich) coated coverslips that were placed on a pre-cleaned microscope slide, and sealed with VaLP (1:1:1 Vaseline: Lanolin: Paraffin). Cells were imaged using a 100 X ELWD Plan APO (NA 1.40 oil) objective mounted on a Nikon TE 200 microscope, with images captured with a Nikon CoolSNAP HQ CCD camera controlled by MetaMorph software (MDS Analytical Technologies). Quantitative morphology analysis of manually thresholded phase-contrast images was performed using the CellTool software program [Lacayo et al., 2007, Pincus and Theriot, 2007, Sycuro et al., 2010] using published protocols [Sycuro et al., 2010]. Centerline data for each strain was obtained from CellTool, imported to MATLAB, and fitted to a generalized sine curve, 

\[ y = R \sin(2\pi x/P + \phi) \]

where \( R \) and \( P \) represent the helical radius and helical pitch of the cell, respectively, and \( \phi \) is a phase term added to allow for an arbitrary origin of the sine function. Cells with non-helical morphologies resulted in poor fitting, characterized by a large sum of squared error (SSE > 0.2), and were removed from the datasets.

In addition to cell shape, flagella morphology was measured for each strain. Flagellum number counts were acquired from SEM (LSH100 and PMSS1 and their respective straight rod mutants) or TEM images (B128 and the flagellar mutants). Flagellum length measurements were acquired from TEM images collected from the same preparation for all strains. TEM images were uploaded to ImageJ and using the segmented line selection tool, a single flagellum length was measured for each cell, one flagellum per cell.

### 3.2.3 Preparation of Polymer Solutions

PGM was isolated from mucosal scrapings of pig stomach epithelium and purified by Sepharose CL-2B column chromatography followed by density gradient ultracentrifugation as described in [Celli et al., 2009]. Lyophilized PGM powder was allowed to reach room temperature before opening tubes to avoid condensation. The powder was weighed and 7.5 mg was dissolved in 400 µL of sterile H₂O to prepare a 15 mg mL⁻¹ solution with bacteria, and 15 mg was dissolved in 800 µL of sterile H₂O to prepare a 30 mg mL⁻¹ solution with bacteria. PGM was allowed to hydrate and equilibrate for 48 hours at room temperature.
before use.

Stock solutions of methylcellulose (MC) from Sigma-Aldrich (M0261) were prepared by making 20 mg mL\(^{-1}\) (wt/vol) solutions in sterile H\(_2\)O, where the mixture was slowly agitated overnight at room temperature using a tube rotator.

### 3.2.4 Particle-tracking Microrheology

The characterize the microrheological properties of each solutions fluorescent polystyrene latex beads (1.001 ± 0.01 \(\mu\)m diameter) (Polysciences Inc.) were added to each sample to a final bead concentration of 0.05% beads by volume in a final volume of 1 mL. In addition, flagellated but nonmotile bacteria (LSH100 \(\Delta\)motB) were grown in liquid broth (BB10) at an O.D. (600) 0.3 - 0.7. These bacteria were added to solutions to produce a 10% bacteria mixture by volume, and bacteria were examined after 45 minutes of incubation in each solution at 37\(^\circ\) C under microaerobic conditions. A 10 \(\mu\)L volume of bead or bacteria solution was then pipetted onto a glass slide with a secure spacer (Secure-Seal, Sigma-Aldrich) and covered with a coverslip. Samples were imaged using an Olympus IX 70 microscope (40 X Plan N, 0.45 NA) with QCAM CCD camera (Qimaging) at 20 fps and 0.2312 \(\mu\)m/pixel pixel size. Fluorescent beads were excited using an Olympus BH2 Mercury arc source while bacteria were imaged using phase contrast with light from a halogen bulb. Focus was set to the center and middle Z-positions of the sample in order to minimize edge effects. Videos were captured at 30 sec intervals using Micro-Manager open source acquisition software [Edelstein et al., 2010] and were analyzed in MATLAB v7.12.0 using a particle-tracking routine that finds the center of intensity of each bead or bacterium using a polynomial Gaussian fit [Rogers et al., 2007]. Beads or bacterium that drifted were de-drifted using a custom MATLAB routine and superfluous tracked objects were removed.

### 3.2.5 Bacteria Motility Measurements

Bacteria were grown in liquid culture broth to an O.D. (600) of 0.5 - 0.7 and kept warm at 37\(^\circ\)C under microaerobic conditions until use. 10 \(\mu\)L of culture was added to 80 \(\mu\)L
of PGM solution and 10 µL of pH 6 buffer (0.1 M phosphate-succinate) to produce a 10% bacteria mixture by volume and the final PGM concentrations used were 15 mg mL\(^{-1}\) and 30 mg mL\(^{-1}\). For methylcellulose solutions, 10 µL of culture was added to a solution that consisted of 50 µL of MC stock solution (20 mg mL\(^{-1}\)) and 40 µL of pH 6 buffer (0.1 M phosphate-succinate), to produce a 10% bacteria mixture by volume and a final concentration of MC at 10 mg mL\(^{-1}\). To produce a 10% bacteria mixture by volume and a final concentration of MC at 15 mg mL\(^{-1}\), 10 µL of culture was added to 75 µL of MC solution (20 mg mL\(^{-1}\)) and 15 µL of pH 6 buffer (0.1 M phosphate-succinate). Bacteria were incubated for 45 min in their respective PGM or MC solutions at 37\(^{\circ}\)C under microaerobic conditions prior to imaging. After the incubation period, each cell suspension was mixed by gentle pipetting and 10 µL was applied to standard glass microscope slides with secure imaging spacers (9 mm in diameter and 0.12 mm depth, Secure-Seal, Sigma-Aldrich). A coverslip was placed over the fluid and securely sealed and samples were immediately imaged at room temperature using a Nikon TE 200 inverted microscope (60X ELWD Plan Fluor, 0.7 NA Phase lens) and videos captured with a Nikon CoolSNAP HQ CCD camera (100 m sec intervals over a 10 sec period (10 fps), 0.109 µm/pixel) using the MetaMorph software (MDS Analytical Technologies). Bacteria were tracked using the Volocity software (v6.1) (Improvision, Perkin Elmer, 2011).

Videos were processed and converted to 8-bit files using ImageJ (http://rsbweb.nih.gov/ij/) and uploaded to the particle-tracking program in Volocity v6.1 to generate tracks based on the centroid position of each object identified (area, 0.05 - 8.0 µm\(^2\)). Individual trajectories were obtained for at least 10 frames of the video (1 sec). Bacteria showing a displacement less than 0.3 µm or a mean squared displacement (MSD) less than 0.1 µm\(^2\) were considered to be immobile and were removed. Individual trajectories of 100 bacteria were acquired, examined visually to ensure accuracy, and imported into MATLAB v7.12.0 for smoothing using a five-point Savitsky-Golay filter to remove noise effects caused by wiggling trajectories [Hyon et al., 2012] and finite tracking resolution [Son et al., 2013]. Stops, reorientations, swimming speeds, and reversals events were segmented and were used to describe \textit{H. pylori}.
motion.

Videos gathered for tracking individual bacterial cells for velocity analysis were used to determine the fraction of motile and nonmotile (immobilized) bacteria in broth and PGM media. We classified all bacteria with displacements less than 0.3 μm as immobile and bacteria with displacements greater than 0.3 μm as motile. The percentage of immobilized bacteria was calculated by dividing the total number of nonmotile cells by the total number of bacterial cells. The total bacterial population included nonmotile bacteria and motile bacteria (bacteria with displacements between 0.3 - 1.5 μm, and bacteria with displacements > 1.5 μm that were processed through MATLAB and segmented to acquire swimming speeds after tracking by Volocity). The number of clumped bacteria (clusters of aggregated bacteria) was analyzed for B128 and the flagellar mutants in viscous PGM solutions (15 mg mL^{-1}). The percentage of clumped bacteria was calculated by dividing the total number of clumps by the total bacterial population. The total bacterial population consisted of the number of clumped bacteria, nonmotile cells (individual cells with displacements < 0.3 μm), and motile cells (as described above).

3.2.6 Statistical Comparisons

When comparing populations the Kolmogorov-Smirnov (K-S) statistics tool in CellTool to assay differences in cell shape morphology, including cell length and side curvature distributions, as described in detail in Sycuro et al., 2010 and 2012. Statistical comparisons between wild-type helical radius, helical pitch, and pitch angle distributions were done using unpaired nonparametric Kolmogorov-Smirnov (K-S) tests in MATLAB v7.12.0. To make statistical comparisons between wild-type vs. mutant instantaneous speed distributions; cell path trajectories; frequency of reversals; and the ratios of forward and reversal swimming speeds, unpaired nonparametric Kolmogorov-Smirnov (K-S) tests were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA USA). For each comparison, a K-S p-value < 0.05 was considered significant.
3.3 Results and Discussion

3.3.1 Microrheology of Polymer Solutions

To examine the microrheological properties of the environment in which *H. pylori* motility is to be measured we used microscopic single particle tracking. This technique probes the Brownian motion of particles and has been previously applied to investigate the microrheology of PGM [Lieleg et al., 2010, Bansil et al., 2013, Georgiades et al., 2014]. To model the viscous environment of the human gastric mucosa where *H. pylori* resides, we used physiologically relevant concentrations of PGM of 15 and 30 mg mL$^{-1}$. For comparison to previous work on *H. pylori* motility in viscous solutions [Hazell et al., 1986, Worku et al., 1999], we also examined methylcellulose solutions at concentrations of 10 and 15 mg mL$^{-1}$. Figure 3.1A shows the averaged mean squared displacement (MSD = $(\langle r(t)^2 \rangle - \langle r(0)^2 \rangle)$, µm$^2$) of 1 µm diameter fluorescent polystyrene particles calculated from the measured position $r(t)$ as a function of time $t$. To confirm that bacteria experience a similar environment, we also tracked ΔmotB mutants, which retain wild-type flagellum structure but have non-functional flagellum motors [Ottemann and Lowenthal, 2002] (Figure 3.1B). We found that the MSD values of non-motile bacteria were smaller relative to those acquired for diffusing particles (Figure 3.1B). This reflects the increased drag due to the larger size and anisotropic shape of bacteria compared to spherical particles.
Figure 3.1: Mean square displacement (MSD, µm²) values as a function of time (sec) for 1 µm fluorescent particles (A) and LSH100 ΔmotB bacteria (B) in broth (BB10), PGM (15 mg mL⁻¹ and 30 mg mL⁻¹) and MC (10 mg mL⁻¹ and 15 mg mL⁻¹) along with (C) Average power of law exponents (α). Average viscosity values (η, cP) and their standard deviations are summarized in the table above (see text for calculation details). All error bars represent standard error and dashed lines represent linear scaling of MSD over time (α=1). *This value is significantly larger compared to the others due to the gel-like nature of PGM at 30 mg mL⁻¹. [Martínez et al., 2016]
By fitting the ensemble averaged MSD to equation 2.11, we obtained the exponent $\alpha$ and $A$. For each solution, the values of $\alpha$ were the same for both polystyrene particles and $\Delta$motB bacteria (Figure 3.1C). Brownian diffusion ($\alpha = 1$) was observed in broth, PGM at 15 mg mL$^{-1}$, and MC solutions of 10 and 15 mg mL$^{-1}$, implying these solutions behave as viscous liquids (Figure 3.1C). The viscosities ($\eta$) (cP) of each solution were calculated using equation 2.10 (Figure 3.1C). Sub-diffusive behavior was observed for PGM at 30 mg mL$^{-1}$ ($\alpha = 0.65 \pm 0.10$), implying that at this higher concentration, PGM is a gel-like viscoelastic polymer. Our results are in agreement with the observations of Georgiades et al., where a transition to viscoelastic behavior was observed in PGM at 25 mg mL$^{-1}$ [Georgiades et al., 2014]. Our results indicate that PGM at 30 mg mL$^{-1}$ displays gel-like properties that resemble the rheological gel-like environment of mucus near the gastric epithelium, and PGM at 15 mg mL$^{-1}$ displays solution-like properties that resemble the rheological environment that $H. pylori$ encounters as it swims through the gastric mucus found near the acidic lumen of the stomach [Taylor et al., 2004]. We used these PGM concentrations in our motility experiments to model the solution and gel-like environments of stomach mucus and to examine $H. pylori$ motility.

3.3.2 Morphological Measurements and Analysis

To address the impact of cell morphology on motility, we began by analyzing the morphology of three unrelated wild-type strains: LSH100, B128, and PMSS. Cell morphology was characterized by analyzing phase contrast images using the CellTool software [Pincus and Theriot, 2007, Lacayo et al., 2007, Sycuro et al., 2010], which has been previously used to segregate $H. pylori$ cells based on different shape parameters, including cell length $L$, diameter $d$, and side curvature [Sycuro et al., 2010, Sycuro et al., 2012, Sycuro et al., 2013]. Side curvature is the measure of the total curvature of a bacterial cell excluding its poles and the curvature itself is defined as the reciprocal of the radius of a circle that is tangent to a curve made at any point [Sycuro et al., 2010]. Wild-type $H. pylori$ strains display heterogeneous morphologies ranging from straight to predominantly helical, and bacterial cells of different
cell lengths were observed within each population (Figure 3.2A). As summarized in Table 3.1, all three strains have similar values of cell diameter but distinct cell length and side curvature profiles.

<table>
<thead>
<tr>
<th>Strain</th>
<th>$L$ ($\mu$m)</th>
<th>Side curvature ($\mu$m$^{-1}$)</th>
<th>$d$ ($\mu$m)</th>
<th>$R$ ($\mu$m)</th>
<th>$P$ ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSH100</td>
<td>3.17</td>
<td>5.26</td>
<td>0.55</td>
<td>0.22</td>
<td>2.3</td>
</tr>
<tr>
<td>B128</td>
<td>2.82</td>
<td>3.08</td>
<td>0.58</td>
<td>0.10</td>
<td>2.4</td>
</tr>
<tr>
<td>PMSS1</td>
<td>3.33</td>
<td>3.10</td>
<td>0.57</td>
<td>0.14</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Table 3.1: Average cell shape parameters for each wild-type strain. [Martínez et al., 2016]

To further characterize helical morphology, we obtained the helical radius ($R$) and pitch ($P$) (length of one complete helical turn) using centerline measurements provided by CellTool. For each bacterial cell, centerlines were fitted using a sine function $y = R\sin(2\pi x/P + \delta)$ (Figure 3.2B). Figure 3.2 C-E show the distribution of each strain’s cell length, helical radius, and helical pitch and their average values are summarized in Table 3.1. All strains showed overlap in cell length distributions, but the B128 population shows a higher probability of shorter cells (Figure 3.2C). PMSS1 showed an increased helical pitch as compared to both LSH100 and B128 (Figure 3.2E), while LSH100 showed significantly increased helical radius as compared to both B128 and PMSS1 (Figure 3.2D and Table 3.1). These observations correlated well with LSH100 having increased side curvature (Figure 3.2A and Table 3.1), while B128 and PMSS1 showed smaller values consistent with a smaller radius.

Flagellum morphologic parameters were also examined by my collaborators using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The three wild-type strains showed a mixed population of cells with 0 to 6 uni-polar flagella (Figure 3.2F). LSH100 and B128 shared a median flagellum number of $3 \pm 1$ ($\pm$ standard deviation), while PMSS1 had a median flagellum number of $4 \pm 1$ and a higher proportion of bacteria with 4 to 6 flagella (55%) relative to LSH100 (34%) and B128 (36%) (Figure 3.2F). LSH100 had a median flagellum length of $3.4 \pm 0.3 \mu$m, which was shorter than both PMSS1
(4.4 ± 0.3µm) and B128 (4.4 ± 0.4µm) (Figure 3.2F).

In summary, the three wild-type *H. pylori* strains examined varied in multiple cell morphologic parameters expected to influence motility, including cell length (B128 shortest), helical radius (LSH100 highest), flagellum number (PMSS1 highest), and flagellum length (LSH100 shortest).
Figure 3.2: (A) Side curvature vs. cell length (µm) for individual bacterial cells LSH100 (blue, n=282), B128 (green, n=274), and PMSS1 (orange, n=222) bacteria. Inset panel: TEM images of each. Scale bar = 1 µm. (B) Representative bacterial centerlines (red dots) for each wild-type strain fitted with a generalized sine function, (black dashed line). (C-E) Probability density for cell length (µm) (C), helical radius (µm) (D), and helical pitch (µm) (E) of LSH100 (n=262), B128 (n=272), and PMSS1 (n=215) bacteria. (F) Percent of the total bacterial population with a given flagellum number (n=104-110 bacteria). [Martínez et al., 2016]
3.3.3 Motility of \textit{H. pylori}

To examine \textit{H. pylori} motility, we used live-cell imaging and particle tracking methods to automatically track hundreds of individual bacterial cells in broth and in viscous PGM or MC media. We collected videos of bacteria swimming at the mid-level plane between the coverslip and the glass slide. We tracked bacteria from ten-second videos, which allowed us to capture bacteria swimming in the field of view and it provided sufficient time to acquire a broad sampling of each bacteriums instantaneous swimming speeds. The recorded videos show that \textit{H. pylori} exhibits a complex motion consisting of periods of straight swimming (runs), interrupted by short periods of directional reorientation and/or reversal in swimming direction. Figure 3.3A shows a representative bacterial trajectory and Figure 3.3B shows its corresponding instantaneous speed as a function of time. The speed is observed to vary between high values ($v \approx 10 - 15 \text{ \mu m/sec}$) and low values of $v \approx 1\text{\mu m/sec}$. The low speeds show a one to one correspondence with reorientation events represented by a large change in swimming direction ($\Delta \theta$) as shown in Figure 3.3C.

Using a similar method to Son et al. [Son et al., 2013] we classified reorientations as any event where abrupt decreases in bacterial swimming speed ($v_{local \ min} < (v_{avg}/2)$) happened or $\Delta \theta$ was significantly larger than angle changes resulting from rotational diffusion ($\delta \theta > 25$ degrees was used exclusively in this study). Reversals were classified as any reorientation event for which the directional angle change $\Delta \theta > 110$ degrees (Figure 3.3A). In our analysis, the direction of swimming observed at the start of the video was considered forward solely as an annotation relevant to the video frame and not relative to bacterial polarity. Polarity could not be determined because bacterial flagella were not observable at the magnification and optical resolution of our experiments. After a reversal occurred, bacteria were assumed to swim in the reversed direction until another reversal event took place. Using these criteria, all trajectories were segmented into forward and reverse swimming directions and reorientation events. From the segmented trajectories, we calculated several motility parameters: instantaneous swimming speeds (the speed between two points of a track); reversal frequency (reversals per second) and speed measurements of both forward
and reverse directions; and percent track linearity %TL, the ratio of average straight line velocity (µm/sec) to average swimming speed (µm/sec) x 100%). Measurements acquired during reorientation events were excluded from our speed calculations.

Figure 3.3: A representative bacterial trajectory depicting the swimming motion of *H. pylori*. (A) A representative bacterial trajectory of *H. pylori* is shown segmented into forward (blue) and reverse swimming directions (red). In our analysis, the direction of swimming observed at the start of the video was considered forward. A reversal in swimming direction (green) was identified when bacteria exhibited a large angle change (Δθ > 110 degrees). Upon a reversal, bacteria were assumed to continue swimming in the reversed direction (red) until another reversal took place. (B) Instantaneous forward and reversal swimming speeds and (C) change in swimming angle (Δθ) of the bacterium in panel A over the course of time it was tracked (X (green) denotes reversals in swimming direction).
Experiments and Analysis of Motility of Wild-Type Strains

As shown in Figure 3.4A-C, the three wild-type *H. pylori* strains exhibited broad overlapping distributions of instantaneous swimming speeds in broth and PGM. These distributions are concatenations of instantaneous swimming speeds from all bacterial cells analyzed for each strain and in each solution tested. The shapes of these broad speed distributions depend on both strain and medium in a complex way, presumably reflecting the differences in cell shape, number of flagella, and flagellum length. To characterize such broad distributions it is not enough to use a single average. We therefore computed the average speed ($v_{avg}$), median speed ($v_m$), max speeds ($v_{max}$), and standard deviation ($\sigma$) of the distribution of speeds as shown in Table 3.2.

![Figure 3.4: Probability density summarizing speed distributions for LSH100, B128, and PMSS1 bacteria swimming in broth (BB10) (A), in viscous solutions of PGM (15 mg mL$^{-1}$) (B), and in gel-like PGM solutions (30 mg mL$^{-1}$) (C)](image-url)
Table 3.2: Table showing for each strains the number of bacteria tracked, $n$, average swimming speed, $v_{avg}$, median swimming speed, $v_m$, maximum swimming speed measured, $v_{max}$, and standard deviation of the swimming speeds, $\sigma$. [Martínez et al., 2016]

<table>
<thead>
<tr>
<th>Solution</th>
<th>Strain</th>
<th>n</th>
<th>$v_{avg}$</th>
<th>$v_m$</th>
<th>$v_{max}$</th>
<th>$\sigma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB10</td>
<td>LSH100</td>
<td>B128</td>
<td>99</td>
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First considering LSH100 and PMSS1, Figure 3.4A-C shows that LSH100 speed distributions are different from those of PMSS1, having lower speeds than PMSS1 in all three solutions. For example, in all three solutions $v_{avg}$ and $v_m$ are almost twice as large for
PMSS1 compared to LSH100 and the standard deviation is approximately 30-40% larger (Table 3.2). Although \(v_{avg}\) of LSH100 and PMSS1 does not vary much between broth and PGM, these strains show increased proportion of higher swimming speeds in PGM compared to broth. This observation is also confirmed by the change in \(v_m\). For LSH100 we find \(v_m = 5.7\ \mu m/sec\) in broth, which increases to \(6.5\ \mu m/sec\) in PGM at \(15\ mg/mL^{-1}\), and exhibits a similar median swimming speed of \(6.4\ \mu m/sec\) in PGM at \(30\ mg/mL^{-1}\) (Table 3.2). B128 shows similar speed distributions to LSH100 in broth and PGM at \(30\ mg/mL^{-1}\) (Figure 3.4A,C), while its speed distribution shifts to higher speeds in PGM at \(15\ mg/mL^{-1}\), becoming more similar to PMSS1 (Figure 3.4B). This may be attributed to that fact that the shape of B128 is similar to PMSS1 but its flagellum parameters are closer to those of LSH100 (3.2F). B128 also exhibits the non-monotonic trend in PGM observed for LSH100 and PMSS1 (Table 3.2).

The speed distributions shown in Figure 3.4 arise from two unrelated factors: (i) variation in bacterial cell shape and size in the population, and (ii) variation in the swimming speed of individual bacteria over time. As seen in Figure 3.3B individual bacteria alter their swimming speed by an order of magnitude over the time of tracking (typically 1-10 seconds). This temporal variation cannot result from variation in cell morphology, which should remain constant during the track time (\(H.\ pylori\) doubling time is 2.5 - 4 hours, depending on the strain, under these growth conditions). In order to separately assess the temporal variation we processed the trajectories of each bacterium and obtained the standard deviation of each bacteria speed, \(\sigma_{bac}\). Figure 3.4D shows the distribution of speed standard deviations, \(\sigma_{bac}\), for the three different \(H.\ pylori\) strains in PGM at \(15\ mg/mL^{-1}\).

The breadth of the \(\sigma_{bac}\) distributions (Figure 3.4D) indicate that population level speed variation (3.4B) is largely due to speed variation within individual bacterial trajectories (as illustrated for an example bacterium in Figure. 3.3B). LSH100, B128, and PMSS1 showed slightly different distributions (K-S statistics, \(p < 0.05\) for all comparisons) suggesting they may have different swimming dynamics in PGM (Figure 3.4D). We obtained similar results when comparing LSH100 to B128 and PMSS1 to B128 in PGM in \(30\ mg/mL^{-1}\),
although these differences were not observed when comparing LSH100 to PMSS1 in PGM 30 mg mL$^{-1}$ ($p = 0.45$). Interestingly, all wild-type strains showed similar distributions in broth ($p > 0.05$). These results indicate that the temporal variation in swimming speeds of *H. pylori* depends on both the strain and swimming environment, and the breadth of the speed distribution is largely due to individual bacteria varying their swimming speed in time.

In addition to monitoring the speed of swimming bacteria, we also recorded the number of bacteria that appeared non-motile for LSH100 and PMSS1 in broth and PGM (Figure 3.5). Bacteria that exhibited displacements $< 0.3 \mu m$ were classified as non-motile (immobile). The percentage of immobilized bacteria in all solutions (10-50%) exceeded the percentage of bacteria with zero flagella (4-9%), and increased with increasing viscosity of the media. PMSS1 had the fewest percent-immobilized bacteria and the highest $v_m$ in all solutions. Thus, the tendency to not be immobilized in the viscoelastic PGM gel correlates with swimming speed.
Figure 3.5: Fractions of immobilized bacteria in viscous PGM media. The percentage of immobilized bacteria was calculated by dividing the total number of nonmotile cells (bacteria that exhibit displacements < 0.3 µm) by the total bacterial population examined, as described in the Materials and Methods (3.2.5). [Martínez et al., 2016]

Motility Comparison of Wild-Type Bacteria to Cell Shape Mutants

To directly address whether the helical cell shape of *H. pylori* impacts its motility, we compared the motility of helical wild-type bacteria to isogenic straight rod mutants (Δcsd6) of LSH100. We found that while the overall speed distribution of the LSH100 Δcsd6 mutant was similar to wild-type LSH100 in broth (Figure 3.6A), $v_m$ decreased by 7% (Table 3.2). In addition, straight rods displayed an 11% reduction in $v_m$ in PGM at 15 mg mL$^{-1}$, and an 8% reduction in $v_m$ in PGM at 30 mg mL$^{-1}$ (Table 3.2). While the reduction in speed between the Δcsd6 mutant and wild-type is statistically significant in the PGM solutions (K-S, $p < 0.0001$), it is not so in the broth solution (K-S, $p < 0.0955$) (Table 3.2).

To further test the generality of this phenomenon, we generated a csd6 deletion mutant in the PMSS1 strain background. In this case, we found significant differences between Δcsd6 mutant and wild-type speed distributions in broth and in viscous PGM solutions.
(Figure 3.6B), with a slightly larger reduction in median swimming speed compared to LSH100 (Table 3.2). For example, straight rods displayed an 11% reduction in $v_m$ in broth and in PGM at 15 mg mL$^{-1}$, and a 13% reduction in a gel-like PGM environment at 30 mg mL$^{-1}$ (Table 3.2).
Figure 3.6: Speed distributions for wild-type and Δcsd6 isogenic straight rod mutants in broth (BB10) and viscous PGM media (15 and 30 mg mL⁻¹) (A,B), and in broth (BB10) and viscous MC media (10 and 15 mg mL⁻¹) (C,D). One of two or three independent experiments are shown. (E,F). Distribution of speed standard deviations, \( \sigma_{bac} \), for wild-type LSH100 (E) and PMSS1 bacteria (F) compared to their respective isogenic straight rod mutants in viscous solutions of PGM at 15 mg mL⁻¹. The distributions show similar speed variation profiles between wild-type and straight rod mutants, where K-S \( p = 0.31 \) for LSH100 vs. LSH100 Δcsd6, and \( p = 0.51 \) for PMSS1 vs. PMSS1 Δcsd6. [Martínez et al., 2016]
To investigate whether straight rods exhibited reduced swimming speeds in a different viscous polymer solution, we analyzed their swimming speeds in viscous methylcellulose (MC) media. In agreement with our results in PGM, we found significant differences in swimming speed distributions (Figure 3.6C,D) and reduced median swimming speeds between straight rods and their respective wild-type strains (Table 3.2). Both straight rods mutants also showed a higher fraction of immobilized bacteria relative to their respective wild-type strains. This effect was most pronounced in PGM at 15 mg mL\(^{-1}\) where a 25 and 40\% increase was observed for LSH100 and PMSS1, respectively. Our results with straight rod mutants indicate that helical morphology enhances the fraction of motile \(H.\ pylori\) and their swimming speed. Motility enhancement was seen in all solutions but did not show an obvious dependence on viscosity.

We utilized the cell trajectory data of the two wild-type and isogenic \(\Delta\text{csd6}\) straight rod mutant strains to investigate whether other motility parameters were affected by perturbation of helical cell morphology. Analysis of the individual bacteria speed standard deviations, \(\sigma_{\text{bac}}\), indicated that the variation in swimming speed in time was similar for the \(\Delta\text{csd6}\) mutant when compared to wild-type bacteria in PGM at 15 mg mL\(^{-1}\) (Figure 3.6E,F) and in broth or PGM at 30 mg mL\(^{-1}\). We did not observe differences in reversal frequency between wild-type strains and their respective isogenic straight rod mutants in broth or viscous PGM or MC media (Figure 3.7A,B). We also calculated the ratios of median forward swimming speed to median reversal swimming speed for individual bacterial cells that reversed and maintained at least 3 instantaneous forward or reversal speed values while swimming. We did not observe any significant differences in the ratios acquired for wild-type strains and their respective isogenic straight rod mutants in any of the solutions tested (Figure 3.7C and data not shown). All strains exhibited relatively similar median ratios, close to 1 (Figure 3.7C).
Figure 3.7: Reversal frequency and ratio of forward to reverse speed for wild-type strains and shape mutants. (A, B) Dot plots summarizing the number of reversals per second of wild-type *H. pylori* strains and isogenic Δcsd6 straight rod mutants in LSH100 (A) and PMSS1 (B) strains in broth (BB10) and in viscous PGM or MC media. Mean values of reversals per second are shown as bolded black lines and error bars indicate one standard deviation from the mean. Data shown is from one of two or three representative experiments for each strain. (C) Dot plots summarizing individual ratios of median forward swimming speed to median reversal swimming speed acquired for each bacterial cell that reversed and maintained at least 3 instantaneous forward or reversal speed values while swimming in broth or viscous PGM solutions (15 mg mL$^{-1}$). Ratios are plotted on a log$_2$ scale and median values are shown as bolded black lines. *K-S p < 0.05 was considered significant and ns = no significant difference. [Martínez et al., 2016]
Finally, we examined percent track linearity (%TL) as a measure of cell path trajectory. Wild-type *H. pylori* strains showed more continuous straight runs in viscous solutions of PGM compared to broth (Figure 3.8A), as previously described (Celli et al., 2009). We did not observe any differences in %TL between wild-type strains and their respective isogenic straight rod mutants in broth and in viscous PGM or MC media (Figure 3.8B,C). Thus, while wild-type *H. pylori* strains show increased %TL in their swimming trajectories in viscous solutions of PGM, this increase in % TL does not require helical cell shape. Our results indicate that while the loss of helical cell shape reduces the swimming speed of *H. pylori*, helicity does not influence any other measured aspect of motility.
Figure 3.8: *H. pylori* % track linearity in viscous PGM media, irrespective of cell shape. Bar histograms show cell path trajectory as percent track linearity (%TL) between wild-type *H. pylori* strains (solid bars, A-C) and isogenic Δcsd6 straight rod mutants (hashed bars, B-C) in LSH100 and PMSS1 strains, as calculated from the ratio of straight-line velocity to curvilinear velocity in broth (BB10) and in viscous PGM or MC media. Error bars represent SD. [Martínez et al., 2016]

Motility Comparison of Wild-Type Strains to Flagella Mutants

We further investigated the effect morphology can have on swimming by comparing the swimming speeds of B128 to two flagella mutants. The B128 sRNA_T mutant has a flag-
ellation profile more similar to PMSS1 (Figure 3.2F and 3.9A). In addition to perturbing flagellum number, we found that both these mutations altered other cell morphology parameters. Both the fliOΔC and sRNA_T mutants displayed increased cell lengths (though similar to PMSS1), and increased cell curvature profiles (but still smaller than LSH100) relative to wild-type B128. These results suggest that perturbation of flagella assembly may influence helical cell morphology and cell length in *H. pylori*.

**Figure 3.9:** (A), Percent of the total bacteria, within a given strain, with a given flagellum number of flagellum (n=83-105). (B), Speed distributions and (C), distribution of bacteria standard deviations in swimming speed, σ_{bad}, for B128 along with flagella mutants, fliOΔC and sRNA_T, in PGM (15 mg mL\(^{-1}\)). [Martínez et al., 2016]

We analyzed the swimming speeds of the different flagellar mutants in PGM solutions at 15 mg mL\(^{-1}\). We were only able to analyze speeds from a small bacterial population
(n=23) for the fliOΔC mutant because of difficulties finding motile bacteria and because cells tended to clump. A positive correlation between flagellum number and swimming speed was observed with significantly reduced swimming speeds (50% reduction in \( v_m \)) for fliOΔC bacteria and increased swimming speeds (19% increase in \( v_m \)) for the sRNA_T mutant (Figure 3.9B and Table 3.3). The B128 sRNA_T mutant also showed a higher \( v_m \) that was 18% faster than PMSS1 in this solution (14.9 \( \mu \)m/sec vs. 12.2 \( \mu \)m/sec) (Tables 3.2 and 3.3). The standard deviation of swimming speeds, \( \sigma_b \), (Figure 3.9C) suggest that sRNA_T displays similar swimming dynamics to wild-type B128 (p =0.60), fliOΔC displays similar swimming dynamics to wild-type B128 (p=0.99), and fliOΔC displays similar swimming dynamics to sRNA_T mutants (p=0.57), thus altering flagellation does not impact temporal behavior in the swimming speed of individual bacteria. We observed a higher fraction of immobilized bacteria (82%) for fliOΔC, but saw a smaller fraction for the sRNA_T mutant compared to wild-type B128 (14% vs. 36%, respectively) (Table 3.3).

<table>
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<th>( v_{avg} )</th>
<th>( v_m )</th>
<th>( v_{max} )</th>
<th>( \sigma )</th>
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Table 3.3: Table for B128 and its flagella mutants showing: number of bacteria tracked, \( n \), average swimming speed, \( v_{avg} \), median swimming speed, \( v_m \), maximum swimming speed measured, \( v_{max} \), standard deviation of the swimming speeds,\( \sigma \), average percent track linearity , % TL, and fraction of immobilized bacteria, % Immobile. [Martínez et al., 2016]

To explore whether variation in flagellum number influences other aspects of swimming, we analyzed reversals, the ratio of forward and reversal swimming speeds, and cell path trajectory for wild-type bacteria and the flagellar mutants (Figure 3.10). We observed minimal effects of changing flagellum number on reversal frequency and the ratio of forward to reverse swimming speeds (Figure 3.9C).
Figure 3.10: Reversal frequency, ratio of forward to backwards swimming speeds, and % track linearity for *H. pylori* and flagella mutants of *H. pylori*. (A) Dot plots summarizing the number of reversals per second acquired for wild-type B128 and isogenic flagella mutants in viscous PGM solutions (15 mg mL\(^{-1}\)). Mean values of reversals per second are shown as bolded black lines and error bars indicate one standard deviation from the mean. The fliO\(_{C}\) shows a significant difference to wild-type B128 (KS, \(p = 0.0114\)). (B) Dot plots summarizing individual ratios of median forward swimming speed to median reversal swimming speed acquired for each bacterial cell swimming in PGM (15 mg mL\(^{-1}\)). Ratios are plotted on a log\(_2\) scale and median values are shown as bolded black lines. (C) Bar histograms showing % track linearity (%TL) for wild-type B128 and isogenic flagella mutants in PGM (15 mg mL\(^{-1}\)). Error bars show one standard deviation from the mean and mean %TL values are summarized in Table 3.3. *K-S \(p < 0.05\) was considered significant and ns = no significant difference. [Martínez et al., 2016]
3.4 Conclusions

While variation in cell morphologic parameters among strains and between species have long been noted, our results provide an in-depth, quantitative analysis of *H. pylori*’s natural variation in helical cell and flagellum morphology, and how these parameters impact *H. pylori*’s motility in viscous environments. Most of the previous studies of *H. pylori* motility provide numbers for only the average or maximum speed. Given the wide distribution of speeds we suggest that conclusions based only on the variation of a single speed parameter with varying external conditions could be misleading as the shape of these broad, asymmetric distributions is not fully described by a single parameter such as the average, thus higher moments of the distribution are required to describe the shape of an asymmetric distribution. Our motility studies of three unrelated wild-type *H. pylori* strains in broth and viscous solutions of PGM reveal the swimming speed distributions reflect both temporal variation in the speed of individual bacteria (Figure 3.4) and morphological variation within the population (Figure 3.2).

The observed broad distribution of swimming speeds was found to be largely due to temporal variation in swimming speed, which we characterized using the distribution of individual bacteriums standard deviation in speed, $\sigma_{bac}$ (Figure 3.4). Wild-type strains exhibited temporal variation that was dependent on their swimming environment, however, loss of helical cell shape or perturbed flagellation did not affect how individual bacteria vary their swimming speed with time. Our data suggest temporal speed variation is not influenced by morphology (Figures 3.7 and 3.8), but can be influenced by bacteria-medium interactions, and may reflect fluctuations due to flagella bundling-unbundling events and/or the stochastic behavior in motor activity.

The speed distributions also vary depending on which polymer solution (and at what concentration) in which the bacteria swims. Of particular interest is the observation that compared to broth *H. pylori*’s swimming speeds are slightly higher in PGM at both physiologically relevant concentrations; the higher mucin concentration corresponding to that in
the mucus close to the epithelial surface, and the lower mucin concentration similar to that in the luminal layer. We observed a non-monotonic variation in \textit{H. pylori} swimming speed and increased proportions of immobile bacteria as the viscosity of PGM increased (Table 3.2 and Figure 3.5). Caldara et al. observed a similar behavior in \textit{Pseudomonas aeruginosa}, where bacteria displayed increased swimming speeds in PGM (although at lower PGM concentrations) [Caldara et al., 2012]. Yeung et al. found that addition of mucin at physiological concentrations promoted the ability of \textit{P. aeruginosa} to exhibit rapid motility across the surface of agar [Yeung et al., 2012]. The physical mechanism responsible for the increase in motility of \textit{H. pylori} in viscous PGM solutions is unclear. Possible factors may relate to molecular interactions of bacteria with mucin and the viscoelastic nature of mucin solutions. Recent studies suggest \textit{H. pylori} may directly bind mucins and glycolipids in gastric mucus [Naughton et al., 2013], and there is a growing body of theoretical work exploring how polymer viscoelasticity alters bacterial swimming [Lauga and Powers, 2009].

In methylcellulose, we observed an increase in swimming speed, at a viscosity of 26 cP, followed by a decrease at a higher viscosity of 76 cP (Table 3.2). This non-monotonic behavior agrees with the previous \textit{H. pylori} motility studies in methylcellulose [Worku et al., 1999], along with other studies of bacteria in viscous polymer solutions [Shoesmith, 1960, Schneider and Doetsch, 1974, Greenberg and Canale-Parola, 1977a, Ferrero and Lee, 1988]. Recent work by Martinez et al. suggests that this non-monotonic behavior may be caused by non-Newtonian shear thinning causing the flagellum to experience a lower effective viscosity than the cell body [Martinez et al., 2014]. We did not probe the length and time scales at which flagella interact with the polymer environment in this study and leave quantifying the shear thinning effects of gastric mucin and methylcellulose on bacterial motility for future work.

To directly address the impact of \textit{H. pylori}'s helical cell body shape on motility, we examined the swimming speeds of isogenic straight rod mutants that had similar flagellum length and number to wild-type bacteria. In PGM solutions, which best mimic the different rheological environments \textit{H. pylori} experiences in the stomach, we observed an 8-13%
increase in speed of the helical bacteria compared to straight rods (Table 3.2 and Figure 3.5). We also observed that helical cell shape increased the fraction of motile bacteria. This effect was most pronounced in PGM concentrations that resemble the mucin concentrations found in the outer mucus layer which *H. pylori* must quickly penetrate to escape from the acidic lumen.

The effect of helical shape on motility has been explored in Spirochete bacteria that have periplasmic flagella and utilize a running wave mode of translational motility distinct from that used by bacteria with external flagella [Dombrowski et al., 2009, Charon et al., 2012]. Spontaneous mutants of Spirochaeta halophila that retain flagella but have lost helical cell morphology show decreases in maximal swimming velocity and a lower minimum immobilizing viscosity (MIV) [Greenberg and Canale-Parola, 1977b]. Interestingly, this study also explored the behavior of related strains and species that varied in their helical parameters, characterized as having tight or loose coils. While they did not observe a correlation between helical pitch and maximum velocity or the viscosity at which maximum velocity occurred, they observed marked variation in the MIV (300-1,000 cP) among strains with tight coils having the highest MIV. While helical parameters were not precisely quantified, their results appear consistent with our observations that helical shape provided a similar increase in swimming speed regardless of the viscosity of the medium or helical pitch angle of the parent strain, as well as a more pronounced effect of helical morphology on the percent of immobile bacteria.

Recently Liu et al. observed that *Caulobacter crescentus*, a bacteria with crescent cell shape and external flagella, enhances its motility by precessing its crescent cell body in a helical trajectory [Liu et al., 2014]. Although these bacteria have a different cell geometry compared to *H. pylori*, their findings are consistent with our observations that the shape of the cell body can enhance flagella-mediated motility. Overall, our findings on the effect of *H. pylori*’s helical cell shape on its motility add to a growing consensus that the bacterial cell bodies may play a larger role in motility than previously thought.

In addition to cell shape, we also measured a significant contribution of flagellum num-
ber to *H. pylori* swimming speed. Increased flagellation (+1) of B128 through deletion of the sRNA_T gene resulted in an increase in median swimming speed (19%) and a smaller fraction of bacteria immobilized in PGM (Figure 3.9 and Table 3.3). The sRNA_T mutant bacteria were also longer than the parent strain B128 (Table S3), suggesting that an increase in flagellum number may enhance flagellar propulsion and overcome the propulsive drag provided by helical bacteria with increased cell length. Work by Mears et al. found an analogous finding for *E. coli*, as cells with increased flagellum number were observed to have increased cell length and a slight increase in swimming speed [Mears et al., 2014]. In another *E. coli* study, Darnton et al. suggested that torque on a single flagellum and the flagella bundle are similar and an increase in flagellum number does not result in an increase in swimming speed [Darnton et al., 2007]. They postulated that torque is dissipated when the peritrichous flagella of *E. coli* have to bend around the cell body to form the flagella bundle [Darnton et al., 2007]. Perhaps consistent with this finding, induction of secondary lateral flagella in the single polar flagellated bacterium *Shewanella putrefaciens* resulted in higher directional persistence and spreading in soft agar, but lower swimming speeds [Bubendorfer et al., 2014]. The lophotrichous flagella of *H. pylori* may lessen this effect, but as of now its flagella bundling behavior has not been explored. Future experiments imaging the flagella of swimming bacteria should shine light on the flagella bundling behavior and the effect altering flagella number has on bundling.

While efficient motility is essential for persistent colonization of the gastric mucus layer by this pathogen to get to its extracellular niche and during turnover of mucus and gastric epithelial cells, our study raises the question as to whether there may be selective pressures in addition to swimming speed that dictate *H. pylori* cell shape and flagellum number. Although both helicity and flagellum number alter swimming speed, it is noteworthy that their alteration resulted in a smaller change in speed (\( \approx 1 \ \mu m/sec \)) than the observed changes in an individual bacterium's speed with time (1-5 \( \mu m/s \)). This seems to indicate that other factor contributing to time variation in swimming speeds, such as chemotaxis, play a more important role in motility than morphological changes. In addition, the growth
of a subpopulation of straight rod and curved cells along with bacteria with zero to two flagella persist in all strains analyzed (this work and Sycuro et al. [Sycuro et al., 2010]). Having cells within the population with fewer flagella or a straight rod shape may play an unknown benefit to \textit{H. pylori} unrelated to motility. Extending our cell-based morphologic studies to infected stomach tissues may begin to reveal whether different morphologies favor different niches within the stomach that include the surface mucus, gastric glands, and epithelial cell surface.
Chapter 4

Restive Force Theory Model of Swimming

4.1 Introduction

In this chapter, results using resistive force theory (RFT) to model the dependence of \textit{H. pylori}'s swimming speed on morphology are presented. The results and figures presented in this chapter include results published in Molecular Microbiology [Martínez et al., 2016] and additional results and discussion not presented in that work.

RFT has previously been used to model swimming of \textit{Caulobacter crescentus} [Li and Tang, 2006] and \textit{Vibrio alginolyticus} [Magariyama et al., 1995] by approximating their bacterial cell body as an ellipsoid and the flagellum as a rotating rigid helix. The bacteria were then assumed to swim in a Newtonian fluid and long-range hydrodynamic interactions were neglected. These simplifications make it possible to derive an analytical expression for the bacterium's swimming speed in terms of the motor torque on the flagellum, and the translational and rotational drag on the cell body and helical flagella bundle. Here a similar methodology to Magariyama et al. [Magariyama et al., 1995] to compare RFT to the motility results discussed in chapter 3.

4.2 Methods

As discussed in section 1.3.1, at low Reynolds number inertia does not play a role in a bacterium’s motion. Because of this, the forces, \(F_c\) and \(F_f\), and torques, \(T_c\) and \(T_f\), on a
bacterium must balance, summarized mathematically by equations 4.1-4.3.

\[ F_c + F_f = 0 \]  \hspace{1cm} (4.1)
\[ T_c - T_f = 0 \]  \hspace{1cm} (4.2)
\[ T_m - T_f = 0 \]  \hspace{1cm} (4.3)

Here \(c, f, \) and \(m\) subscripts denote the cell body, flagellum, and motor, respectively. As described in chapter 2, resistive force theory states that the forces and torques on an object are proportional to the local speed and angular rotation rate of that object, with the constant of proportionality determined by the cell and flagella geometry and the viscosity of the liquid [Gray and Hancock, 1955].

We modeled the cell body of wild-type \(H. pylori\) as a rigid helix while the bacterium’s helical flagellum was assumed to bundle and form a single helix. The total force on a translating and rotating helix is a sum of the translational drag, \(F_{\text{drag}} = -\alpha_pv_h\), and a propulsion force resulting from rotation of the helix, \(F_{\text{propulsion}} = \gamma_p\omega_h\), while the total torque is a sum of the rotation drag, \(T_{\text{drag}} = \beta_p\omega_h\), and translation drag, \(T_{\text{propulsion}} = -\gamma_pv_h\). Here \(\alpha_p, \beta_p, \) and \(\gamma_p\) represent the translational, rotational, and propulsion drag coefficients of a helical cell body, respectively. Using equation 1.4 we can write this in matrix form as equation 4.4.

\[
\begin{bmatrix}
F \\
N
\end{bmatrix} =
\begin{bmatrix}
-\alpha_p & \gamma_p \\
-\gamma_p & \beta_p
\end{bmatrix}
\begin{bmatrix}
v_h \\
\omega_h
\end{bmatrix}
\]  \hspace{1cm} (4.4)

In an analogous way, we model the flagella bundle as a single thin helix attached at one end to the cell body with translational, rotational, and propulsion drag coefficients which we designate as \(\alpha_f, \beta_f, \) and \(\gamma_f\).

Using equations 4.1 - 4.3, we solved for the swimming speed of a helical cell body, \(v_h\), in terms of the motor torque, \(T_m\). The swimming speed can be written as the product of \(T_m\), and a shape factor, \(S_h\), that depends on the cell body and flagellum drag coefficients.

\[
v_h = S_hT_m \]  \hspace{1cm} (4.5)
To calculate $S_h$, we used previously derived expressions for $\alpha_h$, $\beta_h$, and $\gamma_h$ in terms of the cell body parameters: cell length ($L$), helical pitch ($P$), helical radius ($R$), and pitch angle ($\Phi$) [Rodenborn et al., 2013].

\[
S_h = \frac{\gamma_h/\beta_h + \gamma_f/\beta_f}{\alpha_h + \alpha_f - \gamma_h^2/\beta_h - \gamma_f^2/\beta_f} \quad (4.6)
\]

Similarly, the flagellum coefficients $\alpha_f$, $\beta_f$, and $\gamma_f$, can be written in terms of the flagellum helical length ($l$), pitch ($p$), radius ($r$), pitch angle ($\phi$),

\[
\alpha_f = (c_n \sin^2(\phi) + c_t \cos^2(\phi))l \quad (4.10)
\]
\[
\beta_f = (c_n \cos^2(\phi) + c_t \sin^2(\phi))r^2l \quad (4.11)
\]
\[
\gamma_f = (c_n - c_t) \sin(\phi) \cos(\phi)rl \quad (4.12)
\]

Here $C_n$ and $C_t$ are the local normal and tangential force expressions for the helical cell body, and $c_n$ and $c_t$ are the local normal and tangential forces for the helical flagella. For these, we use previously derived expression by Gray and Hancock [Gray and Hancock, 1955].

Using these expressions, the drag coefficients and shape factor were calculated using average cell shape measurements acquired from CellTool (shown in Table 3.1 and Figure 3.2). For wild-type $H.\ pylori$ strains, we used the average helical parameters acquired from fitting the centerlines of each bacterial cell within each wild-type population (Figure 3.2B and Table 3.1). We assumed a flagellum diameter ($d = 0.07 \mu m$), and used the average flagellum length ($l$) acquired measurements done on TEM and SEM images (Figure 3.2). Because the flagellum helical pitch ($p$) and helical radius ($r$) for $H.\ pylori$ have not been measured, we assumed a helical flagellum form similar to that of the bacteria Vibrio alginolyticus, where $p = 1.58 \mu m$ and $r = 0.14 \mu m$ [Magariyama et al., 1995] for all bacteria examined in this study.
To compare RFT’s prediction for how bacteria with helical cell bodies to bacteria with straight rod cells bodies we modeled straight rod mutants of *H. pylori* as having ellipsoidal cell bodies attached to a flagella bundle. For this geometry there are no propulsive terms resulting from the rotation of the cell body, resulting in the force and torque on the cell body depending on its speed and rotation rate according to equation 4.13. Where $\alpha_e$ is the translational drag on the ellipsoid, $\beta_e$ is the rotational drag on the ellipsoid, and $v_e$ and $\omega_e$ are the speed and rotation rate of the ellipsoid.

\[
\begin{bmatrix}
F \\
N
\end{bmatrix}
= 
\begin{bmatrix}
-\alpha_e & 0 \\
0 & \beta_e
\end{bmatrix}
\begin{bmatrix}
v_e \\
\omega_e
\end{bmatrix}
\]  
(4.13)

Using the equations 4.1 - 4.3 the swimming speed and shape factor for these bacteria can be derived, resulting in equations 4.14 and 4.15.

\[
v_e = S_e T_m
\]  
(4.14)

\[
S_e = \frac{\gamma_f/\beta_f}{\alpha_h + \alpha_f - \gamma_f^2/\beta_f}
\]  
(4.15)

For $\alpha_e$ and $\beta_e$ we use previously derived analytic expressions [Happel and Brenner, 1965, Chwang and Wu, 1976]. Here $a$ and $b$ are the major and minor axes of the ellipsoid, which we took to be equal to half the cell body length ($L$) and diameter ($D$), respectively. For straight rod mutants previously measured values for $L$ and $D$ were used [Sycuro et al., 2013].

\[
\alpha_e = \frac{16\pi\eta a^2 e^2}{(e^2 + 1) \ln(\frac{1 + e}{1 - e}) - 2e}
\]  
(4.16)

\[
\beta_e = \frac{32\pi\eta a^3 e^2}{1 - e^2 - 3 \ln(\frac{1 + e}{1 - e})}
\]  
(4.17)

\[
e = \sqrt{1 - \frac{b^2}{a^2}}
\]  
(4.18)

We use these equations in combination with cell shape measurements to calculate how varying cell shape affects the drag, and thus the shape factor, for helical and straight rod bacteria. All numerical calculations and manipulations of these equations were done using MATLAB v7.12.
4.3 Results and Discussion

4.3.1 RFT Predictions for Helical Bacteria

I first examine how the shape factor for a helical bacterium (4.6) depends on a cell’s helical parameters by calculating $S_h$ for a typical $H. pylori$ bacterium with a cell length $L = 3.1 \mu m$, helical pitch $P = 2.5 \mu m$, helical radius $R = 0.15 \mu m$, and cell diameter $D = 0.56 \mu m$. These cell body parameters are the mean cell body measurements acquired for the three wild-type $H. pylori$ strains: LSH100, B128, and PMSS1 (Table 3.1). For the flagella bundle, we used a bundle thickness of $d = 0.07 \mu m$; a flagellum pitch of $p = 1.58 \mu m$ and a helical radius of $r = 0.14 \mu m$; and a flagellum length of $l = 4.1 \mu m$ (the mean flagellum length of all the wild-type strains).

Figure 4.1: Rendering of a helix with pitch ($P$) and helical radius ($R$) and the calculated shape factor ($S_h$) for a bacterium with helical cell body. In each plot, the dependence of $S_h$ is shown for a single cell body parameter $L$ (A), $R$ (B), or $P$ (C) while keeping the other two parameters constant. The parameters that were not varied were maintained at a cell length $L = 3.1 \mu m$, helical pitch $P = 2.5 \mu m$, helical radius $R = 0.15 \mu m$, cell diameter $D = 0.56 \mu m$, flagellum length $l = 4.1 \mu m$, flagella pitch $p = 1.58 \mu m$, flagella helical radius $r = 0.14 \mu m$, and flagella bundle thickness $d = 0.07 \mu m$. The blue shaded regions represent the range (min to max) of the cell shape parameters observed experimentally for all wild-type strains. [Martínez et al., 2016]
Figure 4.1 shows the dependence of $S_h$ on one cell shape parameter at a time $L$, $P$ or $R$, keeping the other two parameters fixed. In the parameter ranges observed for *H. pylori* (shaded blue region), $S_h$ decreases monotonically with increasing $L$ or $R$ (Figure 4.1 A,B), while it exhibits a non-monotonic dependence on $P$ (Figure 4.1C), exhibiting a shallow maximum at $P \approx 3.4 \mu m$ with a very slight decrease thereafter. This maximum corresponds to a speed maximum for bacteria with a pitch angle of approximately $15^\circ$. Our model predicts that in order to have a large $S_h$ value, and hence a faster speed for a given value of motor torque, a helical cell must have a short cell length, small helical radius, and a helical pitch of approximately $3.4 \mu m$.

To compare how the variation in cell shape for the different strains alters their shape factor, we calculated $S_h$ for LSH100, B128, PMSS1, and the B128 sRNA_T flagellar mutant (Table 4.1). For each strain, $S_h$ was calculated using its strains average cell body parameters and the same flagella bundle parameters described above. Table 4.1 shows that $S_h$ for LSH100, PMSS1, and B128 sRNA_T all have similar values, while B128 has the largest $S_h$ value (10% larger than all other strains). This is a result of the small average cell length and helical radius of B128 as compared to the other strains (Table 3.1). These shape factors produce a 10% change in swimming speed if all strains are assumed to have similar motor torques their flagella bundles. While this agrees reasonably well when comparing BB10 to B128 in broth, the differences in swimming speed in PGM at 15 mg mL$^{-1}$ were observed to be much larger than 10%, $\sim 0 - 90\%$. The ratios of shape factors to the ratios of swimming speeds (Table 4.1) suggest that differences in shape factor alone, resulting from each strains unique cell shape, do not fully explain the differences in observed swimming speeds between wild-type strains.
Experimental results presented in chapter 3 also show that *H. pylori* strains vary flagellum number (Figure 3.2F), and flagellum number correlates with swimming speed (Figure 3.9). Based on this, we make the simplest hypothesis that the flagella bundle motor torque, $T_m$, increases proportionally with the number of flagella ($N_f$) in the flagella bundle where $T_m = N_fT_f$ and $T_f$ is the torque produced by a single flagellum. With this modification the swimming speed become a function of both the shape factor and flagellum number, $v_h = (S_hN_f)T_f$. Table 4.1 shows the product of $S_hN_f$ calculated for each wild-type strain and the sRNA_T mutant using their respective median flagellum numbers. The differences in the number of flagella between strains results in a larger predicted differences in $S_hN_f$ (20–30%) among the different strains as compared to the shape factors alone. For B128 and its flagellar mutant sRNA_T the ratio of $S_hN_f$ agrees well with the experimental ratio of swimming speeds, reflecting primarily the change in flagellum number, albeit with a small variation in cell length. The predicted ratio of $S_hN_f$ for B128 and sRNA_T, relative to PMSS1, were within 10% of their measured speed ratios (Table 4.1). However, for LSH100 compared to PMSS1 the predicted ratio of $S_hN_f$ does not agree well with the experiment-

<table>
<thead>
<tr>
<th>Strain</th>
<th>$v_m$ (µm s$^{-1}$)</th>
<th>$S_h$</th>
<th>$S_hN_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSH100</td>
<td>6.5</td>
<td>115</td>
<td>345</td>
</tr>
<tr>
<td>PMSS1</td>
<td>12.2</td>
<td>120</td>
<td>480</td>
</tr>
<tr>
<td>B128</td>
<td>12.5</td>
<td>132</td>
<td>396</td>
</tr>
<tr>
<td>B128 sRNA_T</td>
<td>14.9</td>
<td>116</td>
<td>464</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ratio of $v_m$</th>
<th>Ratio of $S_h ± SE$</th>
<th>Ratio $S_hN_f ± SE$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sRNA_T/B128</td>
<td>1.2</td>
<td>1.9 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>sRNA_T/PMSS1</td>
<td>1.2</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>B128/PMSS1</td>
<td>1.0</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>LSH100/PMSS1</td>
<td>0.5</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

Table 4.1: Median speeds in PGM 15 mg mL$^{-1}$, shape factor ($S_h$), and the product of $S_h$ and median flagellum number ($S_hN_f$). $S_h$ and $S_hN_f$ are in units of $10^3$ µm s$^{-1}$/pN µm [Martínez et al., 2016].
tally measured ratio of speeds, suggesting that this method has limitations in modeling the effects of helical shape.

### 4.3.2 RFT Predictions for Straight Rod Bacteria

To further examine the effects of helical shape on swimming speed, we compared the shape factors of wild-type LSH100 and PMSS1 with their respective isogenic straight rod mutants. Figure 4.2 shows the dependence of the shape factor for an ellipsoidal bacterium, $S_e$, on one cell shape parameter at a time, $L$ or $D$, keeping the other parameter fixed. In the parameter ranges observed for $H. pylori$ Δcsd6 mutants (shaded blue region), $S_e$ decreases monotonically with increasing $L$ or $D$. Figures ?? and 4.2 indicate that varying cell length has the largest effect on the shape factors of helical and ellipsoidal bacteria, and that cells with the shortest cell length have the largest shape factors.

Figure 4.2: Calculated shape factor ($S_e$) for a bacterium with ellipsoidal cell body. In each plot, the dependence of $S_e$ is shown for a single cell body parameter $L$ or $D$ while keeping the other parameter constant. The parameters that were not varied were maintained at a cell length $L = 3.1 \mu m$, cell diameter $D = 0.57 \mu m$, flagellum length $l = 4.1 \mu m$, flagella pitch $p = 1.58 \mu m$, flagella helical radius $r = 0.14 \mu m$, and flagella bundle thickness $d = 0.07 \mu m$. The blue shaded regions represent the range (min to max) of the cell shape parameters observed experimentally for all straight rod mutants.

Table 4.2 shows the median swimming speeds obtained experimentally alongside the calculate shape factor for each strain using their average cell shape parameters. We estimate
the motor torque for each strain by taking the ratio of \( v_m \) to \( S_h \) \( (T_m = \frac{v_m}{S_h}) \). Both wild-type \( H. \) pylori strains were predicted to have larger shape factors than their straight rod mutant indicating that if both strains have the same motor torque the wild-type strain would have faster swimming speeds. In experiments, we observed a maximum wild-type swimming speed increase of approximately 20%, while the predicted increase in shape factor between wild-type and straight rod mutants is approximately 35% for LSH100 and LSH100 \( \Delta \)csd6 and 38% for PMSS1 and PMSS1 \( \Delta \)csd6.

<table>
<thead>
<tr>
<th>Strain</th>
<th>( v_m ) ((\mu\text{m s}^{-1}))</th>
<th>( S_h ) ((\mu\text{ms} \text{e c}))</th>
<th>( T_m ) ((\text{pN nm}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSH100</td>
<td>6.5</td>
<td>(1.2 \times 10^{-1})</td>
<td>57</td>
</tr>
<tr>
<td>LSH100 ( \Delta )csd6</td>
<td>5.8</td>
<td>(9.2 \times 10^{-2})</td>
<td>63</td>
</tr>
<tr>
<td>PMSS1</td>
<td>12.2</td>
<td>(1.2 \times 10^{-1})</td>
<td>102</td>
</tr>
<tr>
<td>PMSS1 ( \Delta )csd6</td>
<td>10.8</td>
<td>(8.7 \times 10^{-2})</td>
<td>118</td>
</tr>
</tbody>
</table>

Table 4.2: Median speeds in PGM 15 mg mL\(^{-1}\), shape factor \( (S_h) \), and estimated torque \( (T_m = \frac{v_m}{S_h}) \) for LSH100 and PMSS1 and their respective csd6 mutants. \( S_h \) is in units of \( \mu\text{m sec}^{-1}/\text{pN nm} \) [Martínez et al., 2016].

To further compare our RFT model to our experimental results, we consider a single strain (LSH100) and calculate \( S_h \) for hundreds of wild-type and csd6 mutants using each cell’s measured cell shape parameters (Figure 3.2 and [Sycuro et al., 2013]) and the strains average flagellar parameters (Figure 3.4F). We then calculated each bacterium’s predicted swimming speed, making the assumption that all bacteria within a strain have the same motor torque which we took to be the estimated torque values in Table 4.2. Figure 4.3 shows the experimental swimming speeds observed in broth for LSH100 and LSH100 \( \Delta \)csd6 (Figure 3.6A) compared to the distribution of swimming speeds predicted by RFT. RFT predicts helical shaped bacteria to have increased swimming speeds compared to an ellipsoidal shaped bacteria with the mean swimming speed of helical shaped bacteria being 40% larger than the mean swimming speed of ellipsoidal bacteria. This is significantly larger than the speed difference experimentally observed for LSH100 and LSH100 \( \Delta \)csd6 in broth,
indicating the model overestimates the influence a helical cell shape has on a bacterium’s swimming speed. Figure 4.3 also shows that the RFT speed distributions are significantly less broad than the experimental speed distributions.

As discussed in chapter 3, the breadth of the experimental speed distribution is caused by both time variation in swimming speeds and morphological differences within the population. As expected, this results in a more broad speed distribution than that predicted by RFT, which only predicts the influence cell shape has on swimming. The relative width of the RFT distribution compared to the experimental speed distribution suggests varied cell shapes within a population has a small effect on swimming speeds. This agrees with the conclusion put forth at the end of chapter 3, in that changes in swimming speed with time are much larger than changes resulting from altered cell shape.

![Smoothed histogram of measured swimming speeds (solid lines) and swimming speeds predicted by RFT(dashed lines) for LSH100 and LSH100 Δcsd6. RFT swimming speeds were calculated for hundreds of bacteria (LSH100 n=262, LSH100 Δ csd6 n=481) using measured cell shape and flagella parameters (Figure 3.2 and data from [Sycuro et al., 2013]). For each strains the flagella torque was taken to be the estimated value in (Table 4.2).](image)

To better understand why RFT predicts a larger increase in swimming speed than
we observed we calculated the drag coefficients for a bacteria with cell body parameters equal to the mean cell body parameters we measured (Figure 3.2 and data from [Sycuro et al., 2013]). Table 4.3 shows the drag coefficients for LSH100 and PMSS1 and their respective straight rod mutants calculated using equations and 4.7-4.9 and 4.16-4.17. While the translational drag terms ($\alpha$) are similar between helical and straight cells, a helical cell shape results in an order of magnitude smaller rotational drag ($\beta$). We hypothesize that this numerical difference is due to the Gray and Hancock local drag expressions inaccurately calculating the local forces on the helical cells. Lighthill proposed an alternative form for the local drag coefficients [Lighthill, 1976]. However, recalculating the drag coefficients using Lighthill’s expressions for $C_n$ and $C_r$ results in similar disagreement between the drag coefficients of a helical and straight rod bacteria. Recent computational work by Rodenborn et al. showed that RFT can overestimate the propulsion provided by a rotating helix and underestimate the rotational drag on that helix, leading to inaccuracies in predicted swimming speeds [Rodenborn et al., 2013].

<table>
<thead>
<tr>
<th>Strain</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSH100</td>
<td>$1.0 \times 10^{-2}$</td>
<td>$5 \times 10^{-4}$</td>
<td>$3 \times 10^{-4}$</td>
</tr>
<tr>
<td>LSH100 $\Delta$csd6</td>
<td>$1.0 \times 10^{-2}$</td>
<td>$2.2 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>PMSS1</td>
<td>$0.9 \times 10^{-2}$</td>
<td>$2 \times 10^{-4}$</td>
<td>$1 \times 10^{-4}$</td>
</tr>
<tr>
<td>PMSS1 $\Delta$csd6</td>
<td>$1.1 \times 10^{-2}$</td>
<td>$3 \times 10^{-3}$</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3: Drag coefficients for wild-type LSH100 and PMSS1 and their $\Delta$csd6 mutants. $\alpha$ is in units of pN/(\mu m sec$^{-1}$), $\beta$ and $\gamma$ are in units of pN\mu m sec.

4.4 Conclusions

To further understand the role cell morphology has on swimming speed, we compared the influence of helical cell shape and number of flagella on swimming speed using a resistive force theory model. Assuming a constant motor torque, the swimming speed of a helical bacteria is predicted to decrease with increasing cell length and helical radius, suggesting
the fastest swimming speeds are obtained by bacteria with small cell lengths and small radii. However, altering helical cell shape led to a relatively small change in the RFT predicted swimming speed, \( \sim 10\% \), in contrast to the large changes in swimming speeds observed experimentally (as much as a \( 90\% \)) for wild-type strains with different shape morphologies. Varying the motor torque using a simple linear dependence of motor torque on flagellum number produced a larger change in swimming speed, 20-30\%, than that produced by only altering cell shape and combining both the effect of cell shape and flagellum number could explain the observed differences in swimming speed for B128 and its flagellar mutant. Comparing the other strains, which differ in both flagellum number and shape, produced mixed results. A comparison of the ratios of RFT predictions for PMSS1 to those of B128 and sRNA\( _T \) showed better agreement with experimental results than predictions comparing PMSS1 to LSH100. While we use assumed a linear dependence of motor torque on flagella number, recent modeling studies of bacteria with different numbers of peritrichous flagella predict that swimming speed increases logarithmically with increased flagellation [Kanehl and Ishikawa, 2014]. A logarithmic dependence gives comparable results, predicting an increase of \( \sim 26\% \) when comparing bacteria with four vs. three flagella, and supports our observations that swimming speed correlates with flagellum number.

We then compared RFT to experimental results for wild-type and straight rod mutants of \( H. pylori \). RFT predicted swimming speeds showed reasonable agreement with experimental swimming speeds given the assumptions made. While experiments with straight rod mutants of \( H. pylori \) show that a helical cell body can result in an approximately 10-20\% increase in speed, RFT predicts a slightly larger speed increase of 30-40\%. By calculating the swimming speed for hundreds of bacteria we were able to compare our experimentally observed speed distribution with that predicted using RFT and only altering cell shape. The speed distributions predicted by RFT were less broad when compared to the experimental speed distributions (Figure 4.3). This agrees with our earlier conclusion that the swimming speed distribution is largely a results of the variation in swimming speeds with time, and cell morphology only results in speeds varying by 10-20\%.
While the RFT model provided insight into the relative effect of cell shape and flagellum number on swimming speed it has inherent limitations. RFT overestimates the speed difference between helical and straight rod cells, suggesting RFT may have challenges accurately calculating the drag coefficients for a helical bacteria. Recent work by Rodenborn et al. showed that RFT can lead to inaccurate predictions for a helical flagellum with helical parameters in the range of \( L > 3P \) or \( P < 6R \) [Rodenborn et al., 2013]. These inaccuracies are due to RFT neglecting long-range interactions of the fluid flow from different parts of the helix. While our calculations for the cell body are outside the cited ranges, \( L/P \approx 1.2 \) and \( P/R \approx 10 \), Rodenborn et al. modeled a very thin helix (helical thickness to helical radius \( D/R \approx 0.06 \)), which was meant to mimic the flagellum. For the \textit{H. pylori} cell body, the helical radius is comparable to the cell thickness \( D/R \approx 2 \), meaning long-range hydrodynamic interactions may play a larger role. In light of this, we suggest exploring the use of a numerical solution of the hydrodynamic equations, such as the regularized Stokeslet method [Cortez et al., 2005].

Our model predictions are also based on cell shape parameters of an average or typical bacterium. However, the average or median speed measured from the speed distribution does not necessarily correspond to the speed of the average bacterium. Hence, the present state of modeling is capable of qualitative predictions, but one has to be careful in drawing detailed quantitative comparisons.

Another aspect absent in our model is medium specific interactions. Our experimental results show medium specific changes in \textit{H. pylori} swimming speed which the RFT model cannot explain. Recently, Spagnolie et al. modeled a rotating helix in a viscoelastic fluid and showed that the swimming speed of the helix can increase or decrease depending on the combination of its helical parameters and the viscoelastic parameters of the medium [Spagnolie et al., 2013]. Magariyama et al. had success modeling bacteria swimming in a polymer solution using a two-viscosity RFT model [Magariyama and Kudo, 2002]. In this model a bacterium’s cell body is assumed to interact with the polymer solution while the size of the flagella results it in only interacting with the solvent, this causes the cell body to experience
the polymer viscosity and the flagella to experience the solvent viscosity. We tested this model against our data in PGM pH 6 under the assumption that *H. pylori’s* cell body experiences the viscosity of PGM and the flagella experiences the viscosity of water. We found the model predicted a swimming speed increase approximately an order of magnitude larger than we observed in our experiments. Recently, Martinez et al. developed an alternative model in which the flagella and cell body experiences different viscosities because of polymer shear thinning [Martinez et al., 2014]. The flagella length scale (∼10 nm) and time scale (1/\(\omega_{\text{flagella}}\) ∼ 10^{-2} seconds) are such that polymer shear thinning can result in an altered viscosity relative to the cell body. PGM at pH 6 is weakly shear thinning and the difference in rotation rates of the cell body and flagella (\(\omega_{\text{cell}}/\omega_{\text{flagella}}\) ∼ 10^{-1}) likely results in the flagella experiencing a viscosity half that experienced by cell body [Celli et al., 2007]. These could explain the increase in swimming speeds we observed for helical bacteria swimming in PGM solutions, however further study is required to make any direct link between swimming speed and swimming medium properties.
Chapter 5

Analysis of Reversal Swimming Mechanism

5.1 Introduction

As described in the introduction (see chapter 1.3.2), bacterial species use a variety of different swimming behaviors. These include the classic run-tumble swimming [Berg et al., 1972], in addition to run-reverse [Mitchell et al., 1996, Barbara and Mitchell, 2003, Theves et al., 2013] and run-reverse-flick behaviors [Xie et al., 2011, Son et al., 2013]. A powerful tool in understanding these behaviors has been the field of statistical physics and use of statistical models. Specifically, random walk models have provided a powerful framework for describing all the swimming behaviors mentioned above [Taktikos et al., 2013].

In this chapter, a more detailed analysis of *H. pylori*’s swimming mechanism is conducted and compared to predictions from a random walk model. Earlier results presented in chapters 3 suggested that *H. pylori* uses a run-reverse swimming behavior, however, these observations were based on individual trajectories and not an analysis of swimming direction. By calculating the turn angle distribution I show that after a reorientation it is most probable for the bacterium to reverse it’s swimming direction. In addition, I obtained the run time distribution for the bacteria and find it is well fit by a gamma function.

Our earlier results also indicated that *H. pylori* has a decreased reversal frequency (Figure 3.7) and increased track linearity (Figure 3.8) in mucin solutions. Results presented in
this chapter show that in mucin solutions *H. pylori* decreases the frequency of reversals and has reduced rotational diffusion. These two factors cause bacteria to swim with increased directional persistence resulting in straighter trajectories. The effect of reversals and rotational diffusion on *H. pylori*’s swimming mechanism is well described by a run-reverse random walk model, and predictions from this model are found to agree with experimental findings.

5.2 Materials and Methods

The materials and methods for culturing and tracking bacteria are described in 3.2.1 and 3.2.3 and analysis of the trajectories was done using the procedures outlined in 2.3.1 and 3.2.5.
5.3 Results and Discussion

5.3.1 Analysis of Run-Reverse Trajectories in Broth

Figure 5.1: Trajectory of *H. Pylori* swimming in broth media along with plots of speed ($v$) and absolute angle change ($|\Delta \phi|$) as functions of time. Red circles indicate times identified to be reorientation events using our trajectory analysis procedure.

Figure 5.1 shows a trajectory for a single bacteria after reorientations were identified. We began further analysis of trajectories like that in Figure 5.1 by calculating three trajectory parameters: the average speed during a run, $v_{run}$, the turn angle after a reorientation event, $\theta_{re} = |\dot{\phi}(t + t_{re}) - \dot{\phi}(t)|$, where $t_{re}$ is the time spent in the reorientation state, and the time between runs, $t_{run}$. For bacteria that were not observed to reorient $t_{run}$ was estimated to be the time the bacteria was tracked, while $v_{run}$ was taken to be the average swimming speed. In this way, the distributions of $v_{run}$ and $t_{run}$ are estimates of the actual run speed and run time distributions. Figure 5.2 shows $v_{run}$, $\theta_{re}$, and $t_{run}$ for over a hundred bacteria trajectories obtained from bacteria swimming in BB10. Average run speeds were found to
be as large as $\approx 20 \mu m/sec$ with the distribution peaking at $\approx 5 \mu m/sec$. The turn angle distribution was found to be highly peaked about $\theta_{re} \approx 180^\circ$ signifying predominantly reversals in swimming direction. The run time probability distribution was found to fit well to a gamma function $P(t, a, \lambda) = \lambda^a t^{a-1} e^{-\lambda t}/\Gamma(a)$ (black line) with shape parameter, $a = 2.0 \pm 0.2$, and rate parameter, $\lambda = 1.5 \pm 0.2$. A gamma run time distribution has been observed for other bacteria using run reversal swimming [Theves et al., 2013] and is different from the exponential run-time distribution observed for run-tumble swimming [Berg, 1993].

![Figure 5.2](image_url)

Figure 5.2: Histograms of $v_{run}$, $\theta_{re}$, and $t_{run}$ for *H. pylori* swimming in broth media (BB10). The black line indicates a gamma function fit to the $t_{run}$ distribution with shape parameter, $a = 2.0 \pm 0.2$, and rate parameter, $\lambda = 1.5 \pm 0.2$

In addition to the probability distributions for the reorientation angle and run speed we examined whether $\theta_{re}$ or $v_{run}$ correlated from one run to the next run. Figure 5.3A shows a scatter plot of the $i^{th}$ reorientation angle, $\theta_i$, versus the $i + 1^{th}$ reorientation angle, $\theta_{i+1}$. Reorientation angles are found to cluster around $\theta_{re} \approx 180^\circ$ indicating that after a reversal the next reorientation is likely to also be a reversal. This implies that *H. pylori* reorients its swimming direction predominantly using reversals.

In figure 5.1 bacteria were observed to sometimes alter their swimming speed after a reversal. To quantify this we examine how the $i^{th}$ run speeds, $v_i$, compared to the next occurring run speed, $v_{i+1}$, for all bacteria (Figure 5.3B). Run speeds are found to vary from one run to the next, with bacteria sometimes altering their run speed after a reversal and other times maintaining a similar run speed before and after a reversal. For slower run speeds ($v_i < 8 \mu m/sec$) there appears to be little correlation between $v_i$ and $v_{i+1}$. 
Faster run speed \( (v_i > 8 \, \mu m/sec) \) appear to be followed by either a similar or smaller run speed \( (v_{i+1} \leq v_i) \). To quantify this further, we calculated the relative difference between consecutive run speeds, \( \delta v \) (5.1).

\[
\delta v = \frac{v_{i+1} - v_i}{v_{i+1} + v_i}
\]  

(5.1)

Figure 5.3C shows the distribution of \( \delta v \) values for all bacteria that reversed. The distribution was found to be broader than expected if bacteria varied their run speed randomly from run to run. Fitting the \( \delta v \) distribution to three Gaussian peaks, we found the distribution could be represented by events during which no run speed change occurred \( (\delta v = 0) \), there was a decrease in run speed after a reversal \( (\delta v < 0) \), or there was an increase in run speed after a reversal \( (\delta v > 0) \). The central peak, describing no change in run speed after a reversal, was found to be the largest of the three peaks. The two peaks corresponding to run speed increases and decreases were found to be symmetrically located at \( \delta v \approx \pm 1/2 \). These peaks signify reversals events when the run speed increases by a factor of three \( (v_{i+1}/v_i = 3) \) or decreases by a factor of a third \( (v_{i+1}/v_i = 1/3) \), respectively.

Figure 5.3: Scatter plot of \( i^{th} \) reorientation angle, \( \theta_i \), versus the \( i+1^{th} \) reorientation angle, \( \theta_{i+1} \), (A). Scatter plot of \( i^{th} \) run speed, \( v_i \), versus the \( i+1^{th} \) run speed, \( v_{i+1} \), (B). Histogram of the relative speed difference \( \delta v \) (C). \( \delta v \) histogram is fit to three Gaussians peaks with a best fit obtained for peaks located at at \( \delta v = 0.05 \pm 0.02, \delta v = 0.51 \pm 0.01, \) and \( \delta v = -0.49 \pm 0.02 \). Data presented for \( H. \, pylori \) swimming in BB10.
5.3.2 Analysis of Run-Reverse Trajectories in Pig Gastric Mucin

We next analyzed *H. pylori* trajectories of bacteria swimming in pig gastric mucin (PGM) solutions of 15 mg mL$^{-1}$ and 30 mg mL$^{-1}$ at pH 6. Figure 5.4 shows $v_{\text{run}}$, $\theta_{\text{re}}$, and $t_{\text{run}}$ for bacteria tracked in broth media (BB10), pig gastric mucin at 15 mg mL$^{-1}$ (PGM15), and pig gastric mucin at 30 mg mL$^{-1}$ (PGM30). Compared to broth media, the fraction of bacteria swimming at higher speeds is larger in PGM solutions; e.g. 30% of bacterial run speeds were larger than 8 $\mu$m/sec in PGM 15 mg mL$^{-1}$ and 33% in PGM 30 mg mL$^{-1}$ as compared to 21% in broth. This agrees with the variation of median speed on mucin concentration reported in chapter 3. In PGM 15 mg mL$^{-1}$ the angle changes between 160°–180° had the highest probability, however, smaller angle changes were observed with increased frequency compared BB10 and PGM 30. In PGM 30 mg mL$^{-1}$ the angle change distribution was similar to BB10, being highly peaked around 180° and did not show the increase at intermediate angles seen in PGM 15 mg mL$^{-1}$, indicating only reversals in swimming direction are observed.

The largest change in swimming behavior between BB10 and PGM solutions was the increased time between reorientation events in mucin solutions. To quantify this we fit each run time distribution with a gamma function with shape parameter $a$ and rate $\lambda$ (Table 5.1). The shape parameter ($a$) of each distribution was relatively similar with the largest fit value coming from the in PGM 30 mg mL$^{-1}$. The rate of reorientations $\lambda$ decreased in mucin solutions relative to BB10, with PGM 15 mg mL$^{-1}$ having the lowest frequency of reorientations.
Figure 5.4: Histograms of $v_{run}$, $\theta_{re}$, and $t_{run}$ for *H. pylori* swimming in BB10 (blue), PGM 15 mg mL$^{-1}$ (red), and PGM 30 mg mL$^{-1}$ (green). Black lines indicate a gamma function fit to the $t_{run}$ distribution with fit parameters summarized in Table 5.1.

Table 5.1: Parameters obtained by fitting the $t_{run}$ distribution with a gamma function $P(t, a, \lambda) = \lambda^a t^{a-1} e^{-\lambda t}/\Gamma(a)$.

<table>
<thead>
<tr>
<th>Solution</th>
<th>$a$</th>
<th>$\lambda$(s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB10</td>
<td>$2.0 \pm 0.2$</td>
<td>$1.5 \pm 0.2$</td>
</tr>
<tr>
<td>PGM 15 mg mL$^{-1}$</td>
<td>$1.8 \pm 0.2$</td>
<td>$0.5 \pm 0.1$</td>
</tr>
<tr>
<td>PGM 30 mg mL$^{-1}$</td>
<td>$2.4 \pm 0.3$</td>
<td>$1 \pm 0.1$</td>
</tr>
</tbody>
</table>

These results qualitatively agrees with our earlier observations that bacteria have decreased reversals in PGM 15 mg mL$^{-1}$ and PGM 30 mg mL$^{-1}$ relative to BB10 (Figure 3.10). The non-monotonic decrease in reorientations with mucin concentration may relate to the non-monotonic increase in swimming speed with mucin concentration we observed in chap-
ter 3. Both results indicate that *H. pylori*’s swimming mechanism depends on the mucin concentration, possibly due to the viscoelastic response in mucin solutions being concentration dependent (Figure 3.1).

5.3.3 Analysis of Run-Reverse Trajectories in Methylcellulose

To test whether our results in PGM were due to the increased viscosity of PGM solutions relative to BB10 we tracked *H. pylori* in viscous solutions of methylcellulose (MC) solutions at 10 mg mL$^{-1}$ (MC10) and 15 mg mL$^{-1}$ (MC15) at pH 6. These solutions have similar viscosities to the PGM solutions used and served to examine whether the change in swimming behavior observed in PGM was caused by its increased viscosity. Figure 5.5 shows the $v_{\text{run}}$, $\theta_{\text{re}}$, and $t_{\text{run}}$ distributions for bacteria in MC compared to BB10. Bacteria in MC 10 mg mL$^{-1}$ were observed to swim significantly faster than bacteria in MC 15 mg mL$^{-1}$ and BB10, and bacteria in both MC solutions were found to have similar angle change distributions to BB10. Run times in the MC 15 mg mL$^{-1}$ where similar to those in BB10, $\langle t_{\text{run}} \rangle = 1.3 \pm 0.1$ seconds in BB10 compared to $\langle t_{\text{run}} \rangle = 1.3 \pm 0.2$ seconds in MC 15 mg mL$^{-1}$, while run times were slightly longer in MC 10 mg mL$^{-1}$, $\langle t_{\text{run}} \rangle = 1.8 \pm 0.3$ seconds.
Our results suggest that in mucin solutions, especially in PGM 15 mg mL\(^{-1}\), \textit{H. pylori} has a markedly different swimming behavior compared to BB10 and methylcellulose solutions. To better understand this we modeled \textit{H. pylori}'s swimming behavior using random walk swimming model.

### 5.3.4 Theoretical Model of Run-Reverse Swimming

The decreased frequency of reversals in mucin solutions compared to broth indicates that \textit{H. pylori} has altered swimming behavior in mucin solutions. To examine how this affects its motility we calculate the mean squared displacement (\(\langle (\vec{r}(t) - \vec{r}(0))^2 \rangle\)), velocity autocorrelation function (\(\langle \vec{v}(t) \cdot \vec{v}(0) \rangle\)), and directional autocorrelation function (\(\langle \hat{e}(t) \cdot \hat{e}(0) \rangle\)) where \(\langle \ldots \rangle\) denotes a time averaging. Our experimental data was composed of bacteria that were observed to reversal during tracking and those that were not observed to reverse during...
Bacteria that weren’t observed to reverse during tracking should only have their swimming motion affected by speed fluctuations and the rotational diffusion of the bacterium. We assumed speed fluctuations were small during a run and modeled bacteria to swim with a constant speed, \( v \). The bacterium’s swimming motion can then be completely characterized by the cell’s rotational diffusion constant, \( D_\theta \), and swimming speed, \( v \). Lovely and Danquist showed that under these conditions the velocity and directional autocorrelation functions are equations 5.2 and 5.3 [Lovely and Dahlquist, 1975] with the mean squared displacement (equation 5.4) obtained by integration of the velocity autocorrelation function.

\[
\langle \vec{v}(t) \cdot \vec{v}(0) \rangle = v^2 e^{-2D_\theta t} \tag{5.2}
\]

\[
\langle \vec{e}(t) \cdot \vec{e}(0) \rangle = e^{-2D_\theta t} \tag{5.3}
\]

\[
\langle (\vec{r}(t) - \vec{r}(0))^2 \rangle = \frac{v^2}{2D_\theta^2} (e^{-2D_\theta t} + D_\theta t - 1) \tag{5.4}
\]

We fit the function \( \langle \dot{e}(t) \cdot \dot{e}(0) \rangle \) calculated from our data with equation 5.3 to obtain rotational diffusion constants for \textit{H. pylori} in BB10, PGM 15 mg mL\(^{-1}\), and PGM 30 mg mL\(^{-1}\) (Table 5.2). These values agree well with the rotational diffusion constant estimated in chapter 2 and are in the range of values measured for other bacteria [Berg, 1993, Saragosti et al., 2012]. When comparing this swimming model to experimental results we took the swimming speed in the model to be the average run speed from the run speed distribution, \( v = \langle v_{run} \rangle \) (Figures 5.2 and 5.3).

<table>
<thead>
<tr>
<th>Solution</th>
<th>( D_\theta ) (rad(^2)/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB10</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>PGM 15 mg mL(^{-1})</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>PGM 30 mg mL(^{-1})</td>
<td>0.11 ± 0.01</td>
</tr>
</tbody>
</table>

Table 5.2: Rotational diffusion constant of \textit{H. pylori} in BB10, PGM 15 mg mL\(^{-1}\), and PGM 30 mg mL\(^{-1}\) obtained by fitting \( \langle \dot{e}(t) \cdot \dot{e}(0) \rangle \) to equation 5.3.
Bacteria that were observed to undergo reversals in their swimming direction were compared to a run-reverse model recently developed by Großmann et al. [Großmann et al., 2015]. In this model bacteria are assumed to swim at a constant swimming speed ($v$) and undergo reversals in their swimming direction. The time between reversals is assumed to follow a gamma distribution with shape parameter $a$ and rate $\lambda$, and bacteria are assumed to undergo rotational diffusion with a rotational diffusion constant $D_\theta$. Under these assumptions Großmann et al. obtain the velocity and directional autocorrection functions (5.5 and 5.6) with the mean-squared displacement (5.7) obtained by integrating the velocity autocorrelation function.

$$\langle \vec{v}(t) \cdot \vec{v}(0) \rangle = v^2 \cos(\lambda t)e^{-(2D_\theta + \lambda)t}$$  \hfill (5.5)$$

$$\langle \vec{e}(t) \cdot \vec{e}(0) \rangle = \cos(\lambda t)e^{-(2D_\theta + \lambda)t}$$  \hfill (5.6)$$

$$\langle (\vec{r}(t) - \vec{r}(0))^2 \rangle = C_1 t + C_2(\cos(\lambda t)e^{-(2D_\theta + \lambda)t} - 1) - C_3 \sin(\lambda t)e^{-(2D_\theta + \lambda)t}$$  \hfill (5.7)$$

$$C_1 = \frac{v^2(2D_\theta + \lambda)}{(2D_\theta^2 + 2D_\theta \lambda + \lambda^2)}$$
$$C_2 = \frac{2v^2 D_\theta (D_\theta + \lambda)}{(2D_\theta^2 + 2D_\theta \lambda + \lambda^2)^2}$$
$$C_3 = \frac{v^2 \lambda (2D_\theta + \lambda)}{(2D_\theta^2 + 2D_\theta \lambda + \lambda^2)^2}$$

In our experimental results we observed an order parameter of $a \approx 2$ (Table 5.1) and take $a = 2$ for modeling purposes. We take $\lambda$ to be the values obtained by fitting the run time distribution to a gamma function (Table 5.1), while the model swimming speed was taken to be the average run speed obtained from our experimental data, $v = \langle v_{\text{run}} \rangle$. The $D_\theta$ were taken to be the rotational diffusion constants obtained above.
Figure 5.6: The mean squared displacement, velocity autocorrelation function, and directional autocorrelation function for all bacteria in BB10, PGM 15 mg mL$^{-1}$ (PGM15), and PGM 30 mg mL$^{-1}$ (PGM30) segmented into populations of bacteria which were observed to reverse and those which were not observed to reverse. The green and blue line indicate comparisons to theoretical random walk described in the text.

Figure 5.6 shows the autocorrelations function obtained from bacteria swimming in BB10, PGM 15 mg mL$^{-1}$, and PGM 30 mg mL$^{-1}$ that were observed to reverse (blue) and those which were no observed to reverse (green). The theoretically predicted autocorrelation functions for the reversing and non-reversing populations are shown as solid lines, blue and green, respectively. The model predictions agree reasonable well with our experimental
observations for both reversing and non-reversing bacteria. The presence of reversals results in decreased autocorrelation functions.

In mucin solutions, both the reversing and non-reversing bacteria have larger autocorrelation functions compared to those in BB10. In addition, the decay time of the autocorrelation functions is increased compared to BB10. These results indicate that the decreased rate of reversals and decreased rotational diffusion constants in the PGM results in slower decorrelations and longer decay times compared to BB10.

It is informative to examine the mean squared displacement as reversal rate and rotational diffusion decrease. The mean-squared displacement is initially ballistic \( \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle \sim (vt)^2 \) and in the long time limit \( t \gg 1/(D_\theta + \lambda) \) the mean squared displacement scales linearly with time with an effective diffusion constant \( D_{\text{bac}}, \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle \sim 6D_{\text{bac}} t \).

The long time diffusion constant is obtained by taking the long time limit of the ratio of the mean squared displacement with time (equation 5.8).

\[
D_{\text{bac}} = \lim_{t \to \infty} \frac{\langle (\vec{r}(t) - \vec{r}(0))^2 \rangle}{6t} = \frac{v^2(2D_\theta + \lambda)}{6((\lambda + D_\theta)^2 + D_\theta^2)}
\]  

Using equation 5.8 we estimated \( H. pylori \) to have an effective diffusion constant in mucin solutions that is approximately twice that in broth media (12 \( \mu \)m\(^2\)/sec in PGM 15 mg mL\(^{-1}\), 8 \( \mu \)m\(^2\)/sec in PGM 30 mg mL\(^{-1}\), and 5 \( \mu \)m\(^2\)/sec in BB10).

While this model provides a reasonable comparison to our experimental results it assumes the same swimming speed before and after reversals, which is not the case for all our experimental data. An improved model without this assumption has been implemented in numerical simulations [Theves et al., 2013] but the form of the autocorrelation functions has yet to be found.

### 5.4 Conclusions

We have examined the swimming behavior of the bacteria \( H. pylori \) in a broth media (BB10), methylcellulose solutions, and pig gastric mucin solutions. Our results show that \( H. pylori \) uses predominantly a run-reverse swimming behavior with the time between re-
versals following a gamma distribution. Bacterial swimming speeds were found to have large variation after reversals with bacteria sometimes maintaining their swimming speed after a reversal while other times increasing or decreasing their swimming speed by a factor of three. Bacteria placed in pig gastric mucin solutions swam with slightly increased swimming speeds and a decreased rate of reversals compared to bacteria swimming in broth. Analysis of the mean-squared displacement, velocity autocorrelation function, and the directional autocorrelation functions showed that bacteria that reversed during tracking could be modeled using a run-reverse model while bacteria that did not reverse were only influenced by rotational diffusion. We found that in mucin solutions bacteria swimming with increased persistence in mucin solutions relative to bacteria swimming in BB10. This increased persistence is a result of bacteria having decreased rotational diffusion and decreased rate or reversals in mucin solutions. In the long time limit, this difference in behavior leads to an effective diffusion constant in mucin solutions that is approximately twice that in normal growth media.

Our finding that *H. pylori* uses a run-reversal swimming behavior adds to a growing amount of literature on bacteria using run-reverse swimming. Recently, Theves et al. observed run-reversal behavior for the perilous bacteria *Pseudomonas putida* [Theves et al., 2013]. They found bacteria altered their swimming speeds by a factor of approximately two after reversals and that the time between reversals was well described by a gamma function probability distribution with shape parameter two. Our observations indicate that *H. pylori* has similar swimming behavior to *Pseudomonas putida*, although we found *H. pylori* primarily maintains a constant run speed after reversals in addition to altering its swimming speed. This difference in speed behavior could be caused by the five-fold difference in observed speeds (*H. pylori* $v_{run}$ $\approx$ 6 µm/sec, *Pseudomonas putida* $v_{run}$ $\approx$ 30 µm/sec).

The decreased reversal frequency of *H. pylori* in mucin solutions is likely related to a physiological benefit it gives the bacteria in the stomach mucus layer. Work by Schreiber et al. found that *H. pylori* uses the pH gradient in the mucus layer to orient themselves [Schreiber et al., 2004]. They observed that in the presence of a pH gradient bacteria
remain close to the stomach tissue surface, and after removal of the pH gradient bacteria moved away from stomach tissue surface, spreading into the central mucus layer. Our observations of increased persistence in mucin solutions are consistent with their findings. Experiments by Howitt et al. found when a drop of low pH solution was placed in a solution containing \textit{H. pylori}, bacteria reversed away from the low pH region [Howitt et al., 2011]. Numerous other chemotaxis experiments have found that \textit{H. pylori} chemotactic away from regions of low pH [Lertsethtakarn et al., 2011]. Bacteria in mucin without chemoattractants may decrease reversals in order to better explore their environment and find chemoattractant gradient. A closer examination of the movies shows that some reversals occur when the tracked bacterium is close to another bacterium, suggesting that bacteria sense each other. This is not surprising as quorum sensing has been observed in \textit{H. pylori} and been found to affect its motility [Rader et al., 2011,Rader et al., 2007]. Future experiments examining how \textit{H. pylori}'s reversal rate is altered in mucin solutions in the presence of other chemoattracts could provide further insight into what role reversals play in infection of the stomach.

Lastly, the mechanism responsible for reversals in multi-flagellated bacteria remains a mystery. However, it is worth noting our observation, and those Theves et al. [Theves et al., 2013], that run times follow a gamma distribution with shape parameter two. This gamma distribution is the theoretical distribution for the time it takes for two Poisson process events to occur. Could this mean run-reverse swimming is actually a two step process? The fact that run-tumble is a single step process [Berg, 1993] and run-reverse-flick is a three step process [Xie et al., 2011] also suggests looking into whether run-reverse can be modeled as a two-step process. Additional experiments imaging the flagella during reversals could shine light on the underlying flagella dynamics of reversals and whether reversals are a single step or two step process.
Chapter 6

Bacteria Motility in Gelatin Solutions and Gels

6.1 Introduction

Gelatin is probably the most studied of all biogel, enjoying a long history of rheological experiments. Gelatin is created from denatured collagen and can be classified as Type A, B, or C depending on the treatment and source. Its gel properties are affected by molecular weight, temperature, pH, and ionic concentration. During gelation gelatin molecules go through a reversible coil to helix transition [Djabourov et al., 1988]. These helices are held together by hydrogen bonding and at the gel point forms a three-dimension network spanning the system. The process of forming the gel network has shown to have many phases with different characteristics.

In this chapter, microrheology and bacterial motility results obtained by tracking micron size beads and swimming bacteria in gelatin solutions are presented, with the goal of assessing the use of gelatin solutions for studying the motility of bacteria as a gel is forming. First microrheology results are presented showing that after a gelatin solution is cooled below the gel point the formation of the gel can be monitored using microrheology. After which results showing how the motility of bacteria is altered as a gel is formed are presented.
6.2 Materials and Methods

6.2.1 Preparation of Gelatin

In order to keep gels fresh, samples were made at the start of the day, used in experiments and discarded. Gelatin (Eastman from calfskin, 230 – 240 Bloom) was mixed with 0.22 µm filtered deionized water and heated to 50° C under agitation. The gelatin was allowed to mix and fully dissolve for at least 30 minutes.

For microrheology experiments, fluorescent 3.0 ± 0.15 µm diameter beads (Polysciences Inc) were added to gelatin solutions to give a bead concentration of 0.01 beads by volume and a gelatin concentration of either 1.5%, 3.0%, or 5.0% wt/vol. A 10 µL volume of the bead-gelatin mixture was then pipetted onto a secure spacer (Secure-Seal, Sigma-Aldrich) and placed between a glass slide and cover slide. The slide was then placed on a temperature-controlled stage (PE94, Linkam Scientific Instruments), which was set to 35° C. The sample was allowed to equilibrate at this temperature for 10 minutes. The sample was then quenched to a final temperature ($T_f$) at 30° C/min.

6.2.2 Bacteria Culturing

For bacteria motility experiments, bacteria were cultured in accordance with chapter 3.2. Once bacteria reached an OD of 0.5 – 0.7 they were added to a warm (37°) gelation solution to produce a 1/10 dilution of bacteria at the targeted gelatin concentration. A 10 µL volume of the gelatin mixture was pipetted onto a secure spacer (Secure-Seal, Sigma-Aldrich) and placed between a glass slide and cover slide.

6.2.3 Particle Tracking Microrheology and Bacteria Tracking

Microrheology experiments were done using an Olympus IX 70 microscope with 60x, 0.6 NA objective, while bacteria motility experiments were done using a 40X, 0.45 NA phase contrast object. Images were captured using a Media Cybernetics CCD camera resulting in a final pixel size of 0.12 µm/pixel. Focus was set to the center of the sample and the middle region between the slide and coverslip to minimize edge effects.
For microrheology experiments, videos were captured every thirty seconds for ten minutes after the sample reached its final temperature $T_f$. For bacteria motility experiments the sample was imaged at $25^\circ C$ for up to an hour. Videos for both sets of experiments were captured using the imaging software StreamPix at a frame rate of 20 fps. Images were analyzed in MatLab using a circular centroid routine, further details on this tracking method can be found in chapter 2.23. Beads or bacteria with superfluous features were rejected from tracking. Beads that were observed to drift had their drift removed by fitting beads displacement to a linear fit to obtain the drift speed, and displacement resulting from this drift speed were then subtracted from the beads displacement.

6.3 Results and Discussion

6.3.1 Microrheology of Gelatin

To characterize the gelation process of gelatin we estimated the time for gelation after a gelatin solution is cooled. Figure 6.1 shows the mean squared displacement calculated every thirty seconds for 3.0 μm beads in a 5% gelatin solution which was cooled from $35^\circ C$ to $26^\circ C$, $25^\circ C$, or $24^\circ C$. At $26^\circ C$ the mean squared displacement was linear in time ($MSD \sim t$) during the entire time the beads motion was examined, indicating that beads exhibited simple Brownian motion and behaved as they would in a liquid environment. At $25^\circ C$ a change in mean squared displacement behavior is observed after seven minutes. During the first 7 minutes beads exhibit Brownian motion $MSD \sim t$ after which subdiffusive behavior $MSD \sim t^\alpha$, $\alpha < 1$ is observed. Similar behavior was found the solution was cooled to $24^\circ C$, however, in this case the onset of sub-diffusive behavior occurred early after approximately 5 minutes. As described early, subdiffusive behavior is an indication that viscoelastic effects maybe present and we hypothesized that the change in mean squared displacement behavior is a result of viscoelastic effects arising from gelation.
Figure 6.1: Mean squared displacements calculated every thirty seconds for 3.0 µm beads in 5% gelatin solutions which have been cooled from 35° C to 26° C, 25° C, or 24° C.
We further analyzed this data by calculating the storage ($G'$) and loss ($G''$) moduli using the generalized Stokes-Einstein equation (2.13). Figure 6.2 shows $G'$ and $G''$ as a function of frequency ($\omega$) for a 5% gelatin solution cooled from 35$^\circ$ C to 25$^\circ$ C. Data is displayed during the time when the transition in mean squared displacement behavior was observed. According to percolation theory $G'$ and $G''$ have power law behavior during gelation, and a critical gel is formed when the power law exponent for $G'$ and $G''$ are the same, ($G' \sim G'' \sim \omega^n$) [De Gennes, 1979]. Fitting our data with a power law we find the exponents to be identical after 8.5 minutes at 25$^\circ$ C at a concentration of 5%, confirming the formation of a gel as the cause for the change in mean squared displacement behavior.

![Figure 6.2: The storage ($G'$) and loss ($G''$) moduli calculated from the mean squared displacement data for a 5% gelatin solution cooled from 35$^\circ$ C to 25$^\circ$ C. Dashed lines indicate power law fit to the 8.5 minute data. Power law exponents for $G'$ and $G''$ were within error with the exponent $n = 0.73 \pm 0.07.$](image)

Microrheology experiments were also conducted in 1.5% and 3% gelatin solutions. Figure 6.3 shows the mean squared displacement for beads in 1.5% and 3% gelatin solutions after 10, 30, and 50 minutes at 25$^\circ$C. Beads in both solutions showed Brownian motion with $MSD \sim t$, indicating that gel had not yet formed. Using the Stokes-Einstein equation we calculated the viscosity of 1.5% and 3.0% gelatin to be $26 \pm 3$ mPa sec and $36 \pm 4$ mPa sec.
after 30 minutes at 25° C. The mean squared displacements of beads in 1.5% gelatin did not change after thirty minutes while the mean squared displacement of beads in 3% gelatin continually decreased with time. In addition, vials filled with 5 mL of gelatin solution at concentrations of 1.5% and 3.0% were left to at 25°C for up to a week. 3% gelatin solutions were found to form a visible gel within hours, while 1.5% gelatin solutions did not form a gel after sitting for a week.

![Graph](image-url)

Figure 6.3: Mean squared displacement for 3.0 µm beads in 1.5% and 3.0% after 10, 30 and 50 minutes at 25° C.
6.3.2 Bacteria Motility in Gelatin Solutions and Gels

The motility of *H. pylori* in gelatin solutions was examined at concentrations of 1.5%, 2.0%, 2.5%, 3.0%, and 5.0%. Figure 6.4 shows a single frame from a movie obtained after tracking *H. pylori* swimming in a 1.5% gelatin solution. Experiments at a concentration of 5.0% yielded no motile bacteria.

![Image of bacteria swimming in gel](image_url)

Figure 6.4: *H. pylori* swimming in a 1.5% gel. Individual bacteria trajectories are overlaid on the image in different colors.

Figure 6.5 shows the number of motile bacteria found up to 30 minutes after bacteria were added to each gelatin solution. Motility was observed to be highly dependent on gelatin concentration with bacteria swimming for upwards of thirty minutes in gelatin solutions with concentrations at 2% and below, while fewer bacteria were found swimming in gelatin solutions with concentrations greater than 2% between 10 – 30 minutes and no bacteria were found swimming after 30 minutes.
Figure 6.5: Number of motile bacteria as a function of gelatin concentration. Data from videos obtained up to 30 minutes after bacteria was added to each gelatin solution.

In addition to fewer swimming bacteria in higher concentration gelatin solutions, we observed bacteria that rotated their cell body but were unable to translate. Figure 6.6A shows an image of a bacterium in 3% gelatin that rotated its cell body but did not translate. We analyzed the rotation of this bacteria by taking the cross-sectional intensity of the bacteria and tracking the minima in the intensity. This allowed for an estimate the rotation rate of the bacterium’s cell body (Figure 6.6B-C). We obtained an estimated rotation rate of $\omega_b = 1 \pm 0.1 \text{ Hz}$. This rotation rate is the same order of magnitude measured for a *H. pylori* bacterium that were stuck in a pH 4 mucin gel [Celli et al., 2009] and is approximately ten times smaller than the rotation rate of a swimming bacteria, $\omega_b \approx 10 \text{ Hz}$.
Figure 6.6: (A) Single image of a bacterium which rotated its cell body but did not translate (white line indicates the intensity profile line used to estimate the cells center). (B) The plot of the intensity profile along the profile line, the bacteria location is estimated as being the location of minima in the intensity. Trace of the minima in the intensity profile as a function of time. (C) The oscillating position of the cells center is a result of cell rotation and gives an estimated rotation rate of $\omega_b = 1 \pm 0.1 \text{ Hz}$.

In gelatin solutions where swimming bacteria were observed, we found the swimming speed depended nonmonotonically on gelatin concentration. Figure 6.7 shows the average swimming speed for motile bacteria imaged after ten minutes of being added to each gelatin solution and cooled to 25°C. The average swimming speed peaked in gelatin solutions at a concentration of 2%, after which the average swimming speed decreased. The average swimming speed in 2% gelatin was found to be approximately three times larger than the swimming in normal broth media. We hypothesize that the formation of a gel in 2.5% and 3% gelatin solutions causes a decreased number of bacteria to swim and decreased swimming speeds.
Figure 6.7: Average swimming speed plotted as a function of gelatin concentrations. Error bars indicate standard error of the mean.

In addition, we also observed bacteria to undergo reversals in swimming direction in gelatin solutions. Figure 6.8 shows the distribution of reorientation angles, $\theta_{re}$, for motile bacteria in each gelatin solution. In 1.5%, 2.0%, and 3.0% the probability distribution is peaked at approximately 180° degrees, indicating reversals in swimming direction. In 2.5% gelatin a wide range of angles were observed. The lack of reversals in 2.5% maybe due to the small number of bacteria found swimming (Figure 6.5).
Figure 6.8: Probability distribution of reorientation angles, $\theta_{re}$, in gelatin solutions at concentrations of 1.5%, 2.0%, 2.5%, and 3.0%.

### 6.4 Conclusions

Our findings indicate that the motility of bacteria in gelatin solutions depend on the concentration of gelatin. Bacteria were found to increase their swimming speed relative to broth media up to a maximum in 2% gelatin solutions, after which their swimming speed decreased with increased gelatin concentration. In addition to decreased swimming speeds, we observed few swimming bacteria in gelatin solutions with concentrations above 2% with some bacteria rotating their cell body but unable to translate. Microrheology results for these solutions show that over the time span we conducted experiments a gel did not form, indicating that the changes in bacteria motility we observed were not a result of bacteria swimming in a gel.

Motility is likely altered due to the viscosity of gelatin solutions. The viscosity of 1.5% and 3.0% gelatin solutions is comparable to the viscosities of the methylcellulose solutions
which we earlier examined *H. pylori* motility in (chapter 3, Figure 3.1 and Figure 3.6). *H. pylori* had faster swimming speeds in both methylcellulose and gelatin solutions with viscosities of 26 mPa sec compared to broth solution with a viscosity of 1mPa sec, and slower swimming speeds in gelatin solution with a viscosity 36 mPa sec and methylcellulose solution with a viscosity of 79 mPa sec. These results agree with other experimental observations that as the viscosity of a polymer solution increases bacteria increase their swimming speed to a peak, followed by a continual decrease in swimming speed with viscosity [Martinez et al., 2014, Magariyama and Kudo, 2002, Ferrero and Lee, 1988, Schneider and Doetsch, 1974].

Martinez et al. have suggested increases in swimming speeds in polymer solutions is caused by the flagella acting as a nano-probe and experiencing a different rheology than the bacteria cell body [Martinez et al., 2014]. In our microrheology experiments, we used a 3µm bead which is similar in size to a bacterial cell body. In addition, the rate at which measurements were obtained (20 Hz) is closer to the rotation rate of the cell body ($\omega_{body} \approx 10$ Hz) than it is to the rotation rate of the flagella ($\omega_{flagella} \approx 100$ Hz). As a result, our microrheology results probe the rheological environment the cell body experiences and the environment the flagella experiences could be different. Microrheology experiments using smaller beads and a more rapid measurement rate could address these limitations and address the question as to whether the rheology the flagella experiences is significantly different from the cell body, and whether it explains why bacteria increase their swimming speeds in polymer solutions.
Chapter 7

Conclusions

In this thesis, several factors that influence *H. pylori* motility were examined. Using several wild-type strains of *H. pylori* along with cell shape and flagella mutants, it was shown that *H. pylori*’s helical cell shape results in a speed increase when compared to cells with a straight rod shape. Interestingly, increasing the number of flagella a bacteria had resulted in a larger increase in swimming speed than altering cell shape. Resistive force theory modeling supported this conclusion by showing that the hydrodynamic difference resulting from varying helical cell shape does not explain the difference in swimming speed that was observed experimentally. In addition, resistive force theory predicts increased swimming speeds for a helical shaped bacteria over a rod-shaped bacteria. *H. pylori*’s swimming behavior is best described as run-reverse swimming. This swimming mechanism is well modeled by a run-reverse random walk model which depends on the swimming speed of the bacteria, reversal rate, and rotational diffusion of bacteria. Lastly, motility results for *H. pylori*’s in polymer solutions containing pig gastric mucin, methylcellulose, and gelatin. Bacteria were found to both increase and decrease their swimming speeds in these solutions depending on the polymer concentration, with bacteria increasing their swimming speed when the viscosity of these solutions was approximately 20-30 times that of water. In addition to changes in swimming speed, a number of mobile bacteria and reversal rate were found to change in polymer solutions. Together these results provide an overview of the factors affecting the motility of *H. pylori* and suggest several avenues worth future exploration.
It is interesting to note that cell shape results in a relatively small change in swimming speed as compared to the variation in swimming speeds resulting from a bacterium’s run-reverse swimming behavior or with the variation of swimming speeds caused by increasing or decreasing flagella number. Previous studies have shown that mice infected with both helical and straight rod strains of *H. pylori* later are found to predominately have only the helical strain [Sycuro et al., 2010]. It is unclear whether the persistence of helical bacteria is a result of their increased swimming speeds or whether helicity may play a yet unknown role in persistence and colony formation. Future experiments exploring this question could answer whether helicity is mainly a result of *H. pylori*’s requirement of motility for colonization or whether other selective pressures also lead to helical cells.

The finding that *H. pylori*’s exhibits run-reverse swimming and can be modeled using a run-reverse random walk model provides additional evidence that bacteria that have multiple flagella from one end of the cell body use run-reverse swimming. There are several unanswered questions regarding run-reverse swimming with the most interesting being what exactly happens to flagella during a reversal and when a bacteria is swimming in reverse. Experiments observing the flagella of swimming have proven difficult, but recently rapid imaging of fluorescently labeled flagella [Mears et al., 2014] and the use high magnification dark-field microscopy [Son et al., 2013] have proven to be effective techniques for imaging the flagella. The use of these tools for observing the flagella during reversals is a priority for better understanding the run-reverse swimming mechanism.

Finally, motility results in mucin, methylcellulose, and gelatin solutions highlighted the complex nature of swimming in polymer solutions. In each solution as the concentration of polymer was increased we observed *H. pylori* to increase its swimming speed to a peak. This behavior has been observed for *H. pylori* [Ferrero and Lee, 1988] along with several other bacteria [Martinez et al., 2014, Magariyama and Kudo, 2002, Schneider and Doetsch, 1974], but its cause remains unclear. Work by Martinez *et al.* has shown that at relatively low polymer concentrations bacterial swimming speeds can increase due to bacteria metabolizing small polymer molecules resulting in an increased proton motor force [Martinez et al., 2014].
In addition, they showed that if the flagella experience a different viscosity than the cell body, possibly due to the flagella causing polymer shear thinning, a bacterium can have increased swimming speeds. Future experiments using dialyzed polymer solutions to test for the effect small polymers have on swimming speed, and microrheology experiments conducted using sub-micron probes (<1µm) and rapid measurement rates (>100Hz) could address whether the above-mentioned effects explain our experimental results.
Bibliography


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Curriculum Vitae

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Education

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• 2012, M.A. Physics, Boston University, Boston, MA, USA

• 2010, B.A. Physics, James Madison University, Harrisonburg, VA, USA

Research Experience

• Sep 2011 - Present, Research assistant, Boston University, Conducting research on how cell morphology affects the swimming behavior of the bacterium Helicobacter Pylori. Project responsibilities include microscopy and microfluidic experiments, data analysis and coding, and hydrodynamic modeling.

• Sep 2007 - May 2010, Research assistant, James Madison University, Fabricated microelectromechanical systems (MEMS) to study the torsional properties of multiwalled...
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Teaching Experience

• Mar 2013, Instructor, Center for the Integration of Research, Teaching, and Learning, Developed and taught an online module on Teaching-as-Research using edX. Created online content including presentation, quizzes, videos, and hosted an online discussion lecture.

• Sep 2012 - Aug 2013, Teaching Fellow Peer Mentor, Boston University, Developed and implemented a graduate student peer-mentoring program in which experienced graduate students mentor first time teaching graduate students.

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• Sep 2012 - May 2013, Teaching-as-Research, Boston University, Researched the effectiveness of group worksheets as a teaching tool in peer discussion classes. Outline research project, designed assessments, and gather and analyzed student data.

• Sep 2010 - Aug 2011, Teaching assistant, Boston University, Taught three semesters of introductory undergraduate physics. Responsibilities included creating and implementing group discussion worksheets within the course design and teaching three to five recitation sections a week.

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Awards

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  *Influence of Cell Shape on the Motility of Helicobacter Pylori*

  *Motility of Helicobacter Pylori in Gels*

- March 2012, American Physical Society Spring Meeting, Boston, Massachusetts.  
  *Gelation Kinetics of Gelatin Using Particle Tracking Microrheology*

- April 2010, National Conference for Undergraduate Research, Missoula, Montana.  
  *Fabrication of Multiwalled Carbon Nanotube Oscillators for the Study of Torsional Properties*

**Local Conference**

- Mar 2014, Sixth Annual Instructional Innovation Conference, Boston, Massachusetts.  
  *Developing Reflective Teaching Practices through a Graduate Student Peer Mentoring Program*
- 2011 - 2014, New England Complex Fluids Workshops, Boston, Massachusetts. Sound bites on various research topics.

**Publications**

