

***Helicobacter pylori* moves through mucus by reducing mucin viscoelasticity**

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Abstract

The ulcer-causing gastric pathogen *Helicobacter pylori*, is the only bacterium known to colonize the harsh acidic environment of the human stomach. It is well established that *H. pylori* accomplishes this by production of the enzyme urease, which catalyzes hydrolysis of urea to yield ammonia thus elevating the pH of its environment. However, the manner in which *H. pylori* is able to swim through the viscoelastic mucus gel that coats the stomach wall remains poorly understood. Previous rheology studies on gastric mucin, the key viscoelastic component of gastric mucus, indicate that the rheology of this material is profoundly pH dependent, transitioning from a viscous solution at neutral pH to a gel in acidic conditions. Bulk rheology measurements on porcine gastric mucin (PGM) show that pH elevation by *H. pylori* in the presence of urea induces a dramatic decrease in the viscosity and elastic modulus. Microscopy studies of the motility of *H. pylori* in gastric mucin at acidic and neutral pH in the absence of urea show that the bacteria swim freely at high pH, and are strongly constrained at low pH. Using two-photon fluorescence microscopy to image the bacterial motility in an initially low pH mucin gel with urea present we show that the gain of translational motility by bacteria is directly correlated with a rise in pH indicated by BCECF, a pH sensitive fluorescent dye. Our study shows that the common perception of the helical bacterium moving in a corkscrew like manner through a viscoelastic gel is wrong.

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Introduction

Helicobacter pylori is a spiral shaped, Gram-negative microorganism, that colonizes the human stomach, a highly acidic environment that is not suitable for any other known microorganism (1-5). It is well known however, that *H. pylori*, which is a neutrophile, is able to survive by producing large amounts of the enzyme urease, which hydrolyzes urea present in the stomach to NH_3 and CO_2 , thus elevating the pH in the vicinity of the bacteria to neutral as necessary for survival (6). There is some controversy surrounding the exact mechanism of the urea hydrolysis, but it is generally thought that urea is taken up through a proton gated channel and hydrolysis takes place inside the bacterium buffering the cytosol and periplasm and creating a thin neutral layer around the outer surface. In addition to its ability to elevate pH of its environment, *H. pylori* further protects itself by swimming through the protective layer of gastric mucus in the stomach and attaching to the epithelial cells beneath, where it can cause inflammation over the course of lifelong infection. Both of these factors, the production of urease, and motility, are crucial to the survival of *H. pylori* as it has been shown that neither urease-deficient mutants (7), nor non-motile mutants (8) are able to successfully colonize the stomach (8). However, despite motility being vital for its survival, the actual mechanism by which *H. pylori* swims through the mucus layer is poorly understood

Several early studies examining the rheological properties of mucus secretions from various mammalian stomachs have established that the gastric mucus layer forms a soft viscoelastic gel, with the elastic component of the frequency-dependent viscoelastic shear modulus, $G'(\omega)$ higher than the viscous component, $G''(\omega)$ over a wide range of oscillatory shear frequencies ω (9-14). Given that the mucus layer exhibits a gel-like rheological response, the question is raised of how a flagellated microorganism such as *H. pylori* is able to swim in such

an environment. Although previous studies have examined the motility of *H. pylori* and other bacteria in fluids with varying viscosity (15-18), we are not aware of any studies that indicate the ability of a flagellated prokaryote to swim through a gel with $G'(\omega) > G''(\omega)$. In a recent theoretical investigation on the motility of sperm in viscoelastic media it was shown that swimming speed will decrease with increasing viscoelasticity (19). It has been shown however, that gastric mucin, the glycoprotein content of mucus primarily responsible for its viscoelastic response, undergoes a reversible pH-dependent sol-gel transition from a viscous polymer solution to a soft gel as pH is lowered below pH ~ 4 , and vice versa as the pH is raised (20, 21). This feature of mucin is believed to play a crucial role in its protective function in the stomach in that by forming a gel at low pH, it forms a viscoelastic barrier protecting the lining of the stomach from damage by its own acidic secretions and other insults (22). In fact, the pH of the mucus layer itself is regulated by secretion of bicarbonate ions from the epithelium such that a pH gradient is maintained across the mucus layer from pH 2 in the gastric lumen to approximately neutral pH at the epithelial surface (23, 24). *H. pylori*, has been shown to exhibit a pH tactic response toward elevated pH (25), consistent with the established picture of a bacterium swimming through the mucus layer toward the epithelial surface. However, this still does not explain the manner in which it is motile at all in the low pH mucin gel. In this study we propose to combine the knowledge of the pH-dependent rheological properties of mucin, with the pH-elevation by *H. pylori* to provide an explanation of how this bacterium is motile in the mucus layer. The fundamental hypothesis of this study is that *H. pylori* does not generate sufficient motor torque to be able to propel itself through a viscoelastic mucus gel in the absence of a means to modify the mucus viscoelasticity. However, the urease induced pH elevation of *H. pylori* triggers the transition from gel to sol of gastric mucin and enables the bacteria, which would otherwise be immobile, to swim freely through the mucus.

RESULTS

***H. pylori* and Mucin Rheology**

To examine the impact of *H. pylori* on the bulk viscoelastic properties of gastric mucin and to test the hypothesis that the pH-elevation due to *H. pylori* urease activity may have a crucial impact on mucin viscoelasticity, rheological tests were performed on samples of PGM prepared with a reasonable physiological concentration of 5mM urea and driven down to an initial pH of 4 by addition of hydrochloric acid. Samples were then either incubated with *H. pylori* in or identically prepared as a control with no bacteria present but equal PBS volume added. In samples with *H. pylori* present pH was elevated to values of 7.1 to 7.4 while the control sample remained constant at its initial value of 4.0. This effect was reproduced several times in test samples of H₂O with bacteria and urea.

In these rheological tests, the contrast between these two samples is strikingly consistent with that observed in the pH 4 and pH 6 buffered PGM samples previously published (20). Frequency dependent viscoelastic moduli plotted in Figure 1a exhibit a dramatic drop in the values of both $G'(\omega)$ and $G''(\omega)$ and a change from storage dominant to loss dominant response indicative of the transition from gel to solution in the sample with bacteria present, in direct parallel with the difference between pH 4 and pH 6 buffered samples previously observed. In Figure 1b, the characteristic yield stress and “weak strain overshoot” as described by Hyun et al (26), is observed in the control sample but has completely vanished in the sample with *H. pylori* which, like the previously published pH 6 (solution) sample, has a very limited linear response regime below 0.1 Pa. Finally, the dramatic loss of viscosity and diminishing of shear thinning behavior due to *H. pylori* seen in Figure 1.c is also consistent with analogous tests on buffered samples. These studies present evidence that the pH-dependent gelation of gastric mucin, while perhaps essential as a protective mechanism in healthy stomachs, may also play a

crucial role in the physiology of *H. pylori* infection. The degradation in viscoelasticity of PGM in the presence of *H. pylori* observed here is consistent with the measured change in pH produced by the bacteria in every rheology test examined.

***H. pylori* motility studies**

Further studies were conducted to examine the motility of *H. pylori* suspended in both gel (pH 4) and sol (pH 6) states of PGM. At pH 6 bacteria were observed to qualitatively swim freely, typically moving in circular trajectories with radii on the order of 20 μm as shown in Figure 2. Using particle tracking methods it was possible to analyze several representative trajectories from image sequences to calculate a swimming speed (equivalent to what is referred to as curvilinear velocity in the literature) to be $27 \pm 5 \mu\text{m/s}$. It was also noted that, while swimming, the cell bodies undergo conformational changes that may also be important to motility. In contrast, the cells in the gel phase PGM were clearly elastically confined, undergoing displacements on the order of hundreds of nanometers only, despite the fact that high resolution (100X) images of single cells shown in Figure 3 exhibited rapid rotation of the helical flagella bundle and non-Brownian driven oscillations of the body.

Further analysis of high resolution sequences of images facilitated measurement of morphological parameters of the flagella bundle and dynamics of the bacterial cell body in this confined situation. In Figure 3 several consecutive frames from a 45 second high resolution movie examining the micron length scale conformational changes of a confined bacterium are shown. From this, the helical pitch, p , and radius, r , of the flagella bundle were measured to be 2.1 μm and 0.57 μm , respectively, and the length, L , of the flagella to be 3.17 μm . In the case of *H. pylori*, the fact that the multiple flagella bundle together proves to be a convenience for microscopy since individual $\sim 25 \text{ nm}$ filaments would not be resolved. The thickness of the bundle is difficult to determine by optical microscopy, but assuming several flagella form a

bundle, which when cylindrically packed is approximately 2.5 times the thickness of a single filament, ~ 60 nm is a reasonable estimate, and is the value for this parameter used in the calculations below.

During the time of observation of the bacterium for which snapshots are shown in Figure 3, both the cell body and flagella bundle initially oscillated at a low frequency but later accelerated dramatically to a peak value before slowing down again. Analyzing the time dependent motion in this sequence it was possible to measure the frequency as a function of time for both the oscillatory motion of the cell body, measured by particle tracking, and the flagella bundle itself. As seen in Figure 4 there is a 1:1 correlation, indicating that the oscillations of the cell body, which are in fact two dimensional projections of the rotation of the curved asymmetric cell body shape, are purely driven by rotation of the flagella in combination with the restoring force of the medium. The abrupt fluctuation in oscillation frequency observed in this example was also typical of other cells in the pH 4 PGM. It is unclear whether this behavior is caused by a local spatial variation in chemoattractant concentration driving the motor to turn more rapidly, or if fluctuations in local shear rate are part of a passive modification of the material properties of the bacterium's non-Newtonian environment as discussed in a recent study on locomotion in a viscoelastic environment (27).

Flagella and cell body frequency can further be used to solve for motor torque by the methods of Magariyama *et al.* independently from both information about the cell body dynamics and the flagella dynamics (28-30). In the former case:

$$T_m = \beta_c \omega_c \quad (1)$$

where ω_c is the rotation rate of the cell body (measured above), and β_c is the rotational drag coefficient of the cell body (which is assumed to be a prolate ellipsoid with width $2a$ and length $2b$ in a liquid of viscosity η) and is given by:

$$\beta_c = 8\pi\eta a^3 \left[1 - \frac{3}{5} \left(1 - \frac{b}{a} \right) \right]. \quad (2)$$

Alternatively, a similar expression can be written for the motor torque for flagella rotation information in terms of the rotation rate of the flagella, ω_f , the flagella rotational drag coefficient, β_f , the translational velocity of the cell body, v_c , and a factor for the ratio of the propulsive force to the rotation rate of the flagella, γ_f :

$$T_m = \beta_f \omega_f - \gamma_f v_c. \quad (3)$$

In the present experiments, bacteria are stuck, so $v_c = 0$ and the second term vanishes (hence it is superfluous to define the factor γ_f here). The rotational drag coefficient of the flagella is given in terms of the viscosity of the fluid and the pitch, p , radius, r , length, L and cross sectional diameter, $2d$ of the flagella by:

$$\beta_f = \frac{2\pi\eta L}{[\ln(2p/d) - 1/2](4\pi^2 r^2 + p^2)} (4\pi^2 r^2 + 2p^2) r^2 \quad (4)$$

Using an estimate of $\eta \sim 1$ Pa-s (Figure 1c) , and experimental measurements of $a = 0.45 \mu\text{m}$ and $b = 2.15 \mu\text{m}$ in Equation 2 gives $\beta_c = 7.5 \times 10^{-18} \text{N m s rad}^{-1}$. Inserting this value, and the frequency data above in Equation 1 yields an average torque value of $1.4 \times 10^{-17} \text{N m}$, with a peak torque at the maximum rotation rate of $5.3 \times 10^{-17} \text{N m}$. Conducting the independent calculation of motor torque from Equation 3 (where in this case, $\omega_c = \omega_f$ from Figure 4) and using the geometrical factors for the flagella quoted above in Equation 4 yields an average motor torque of $3.6 \times 10^{-18} \text{N m}$ and a peak torque for the times probed of $1.3 \times 10^{-17} \text{N m}$. It is generally agreed that the calculation of motor torque from the flagella data is more robust

because the flagella geometry is well described as a helix whereas the modeling of cell body shape requires somewhat more of an idealization. Nevertheless, the independently derived values here are within a factor of three. In either case, these torque calculations should be treated as rough estimates given that the hydrodynamic drag formulae above do not account for many aspects of the behavior of gastric mucin previously described, including elasticity, shear thinning, and non-linear response above a yield stress of ~ 10 Pa.

Two-photon fluorescence microscopy

Finally, to tie together the observations from the rheology studies and the preliminary motility studies described above, bacteria were added to PGM samples with HCl added to drive down the (initial) pH to approximately 2, and a 5mM urea concentration. Although initially confined by the gel as discussed above, after approximately 40 minutes bacteria were swimming as if in pH 6 PGM. To reinforce this observation, 2-photon fluorescence imaging of PGM with *H. pylori* in the presence of pH-sensitive dye, BCECF (Invitrogen, Carlsbad, California, USA) was conducted. The use of BCECF, which increases in fluorescence emission as pH rises from acidic to neutral, allowed for direct observation of the pH elevation while observing the bacteria themselves. The washed bacteria were also separately labeled using BacLight (Invitrogen, Carlsbad, California, USA), a fluorescent dye to stain bacterial cells so that the cells could be imaged separately from the fluorescence background. The narrow depth of focus made possible by the use of two-photon fluorescence excitation from a high speed laser scanning source facilitated imaging bacteria without washout from out-of-plane BCECF fluorescence. In Figure 5, three representative frames from this sequence of images of bacteria in a PGM gel initially at pH 2 are shown. After 3 minutes, the bacteria, which appear as bright spots against the initially non-fluorescent background are still stuck in approximately the same place. However, after approximately 20 minutes there has been a significant increase in pH as shown by the increase in fluorescence, and correlated with the increased motility of the bacteria

which have begun swimming and are clearly in different positions in the images. After approximately 40 minutes the fluorescence from the BCECF begins to saturate the image and the bacteria are no longer visible. In Figure 5d overall BCECF intensity, as determined by taking the average pixel values from regions of images free of bacteria, is plotted against time. The kinetics were found to vary slightly in different samples with varying initial pH values, but always exhibited the same qualitative behavior with a monotonic rise in fluorescence intensity in correspondence with the pH elevation. This observation is consistent with previous studies on the kinetic properties of *H. pylori* urease (31).

DISCUSSION

The central result of this study, summarized schematically in Figure 6, indicates that pH elevation by *H. pylori*, long known to be vital to its very survival in the stomach, is also vital to the capability of this microorganism to swim through the mucus layer. We demonstrate novel roles of the enzyme urease, a hallmark of *H. pylori* infection, in two distinct contexts. First, by hydrolysis of urea to elevate the pH of its environment, *H. pylori* reduces the elasticity in the mucin gel, thus modifying their environment from the conditions in which they are initially elastically confined and unable to translate, to the conditions of a neutral pH PGM solution in which they swim freely and are able to penetrate the mucus layer and attach to epithelial cells. Second, the dramatic reduction in the viscoelasticity of mucin via pH elevation from urea hydrolysis may be important to understanding how *H. pylori* impacts the integrity of the mucus layer and compromises its role as a protective viscoelastic barrier. Taken together this presents a picture of a dynamic system in which the bacterium directly influences the rheological properties of the host mucus, which in turn determines the motility of the bacterium.

It is important to recognize that the impact of the bacteria on mucin rheology presented here may in fact be the combined effect of the pH dependence discussed here, and possible

proteolytic digestion of PGM due to other enzymes produced by *H. pylori*. Indeed the breakdown of gastric mucin by *H. pylori* has been previously examined by others, but with apparently contradictory results. Early in vitro studies suggest that *H. pylori* directly compromises the mucus layer by proteolytic degradation of mucin glycoproteins (32, 33). In later studies however, other researchers conclude that an observed loss of high particle weight glycoprotein in mucus from ulcer patients is not the result of proteolytic enzymes, but perhaps from a carbonate-bicarbonate buffer at the mucosal surface due to the hydrolysis of urea (34). In another study comparing endoscopic biopsy samples of patients with and without *H. pylori* infection, one group concludes that although *H. pylori* causes structural changes in the mucus layer *in vivo*, it does not cause a major overall compromise of the barrier (35). Finally, in studies by another group comparing the viscosity of mucus from a small a group of infected patients, untreated subjects, and those after eradication, it is contested that *H. pylori* infection does not reduce the viscosity of gastric mucus (36). However, given the strong correlation with the sharp rise in pH in the presence of *H. pylori* and the subsequent change in rheology in nearly exact parallel with the pH dependence of every measure of viscoelastic response previously established (20), the results here indicate that the dominant effect of *H. pylori* on gastric mucin rheology is that due to pH elevation. Small differences in the exact values of the frequency dependent moduli presented here with those of buffered sample of the identical pH reported previously are likely linked to the very different preparation of the samples. For example, the use of HCl rather than buffer to vary pH results in a different final ionic strength, a parameter which has also been previously reported to impact mucin rheology (20, 37, 38).

In this study the fact that bacteria are physically trapped by the mucin gel provided us with an interesting opportunity to examine their dynamics and facilitated calculation of motor torque by two independent methods. To examine the magnitude of the best estimate of torque of $3.6 \times 10^{-18} \text{ N m}$ in relation to this yield stress, one can divide the by the approximate swept

volume of the flagella, $V = \pi r^2 L = 3.2 \mu\text{m}^3$, to obtain a stress of $\sim 1 \text{ Pa}$. This indicates that the magnitude of torque exerted here is within the linear response regime of the mucin gel.

However, further microrheology studies examining the breakdown of the linear response regime of mucin gels are warranted to determine if the material would yield at the same stress on the micron length scale probed here, as it does in the bulk.

Using the flagella frequency derived value as the basis for comparison, this average torque of $3.6 \times 10^{-18} \text{ N m}$ is roughly an order of magnitude higher than measurements by Tang *et al.* for *Caulobacter Crescentus* ($3.5 \times 10^{-19} \text{ N m}$) (39), and roughly a factor of three higher than torque measured by Reid *et al* for *E. Coli* ($1.3 \times 10^{-18} \text{ N m}$) (40). This is consistent with Tang's hypothesis that the low motor torque and high swimming efficiency of *C. Crescentus* are necessary for survival in the extremely nutrient-poor fresh water environments it has adapted to survive in. In contrast, *E. Coli* and *H. pylori* inhabit the nutrient rich human gut and do not require efficiency of flagella rotation for survival. In the case of *H. pylori*, the generation of an extremely high torque may be important to twist the flagella hard enough to move through mucin at moderately low pH values.

It is interesting to note that the pH elevation observed in the fluorescent images in this study does not appear to be a local effect in the immediate vicinity of a given bacterium, but rather a global effect over the entire sample. This is likely due to the extremely rapid diffusion of the NH_3 molecules, however, this *in vitro* model system does not reflect the full dynamics of the mucus layer in vivo in which active secretion of acid and bicarbonate occur continuously. It was also noted in these images that the bacteria themselves became brighter as time went on suggesting that some of the BCECF passes through the cell wall into the interior of the bacterium. This may be consistent with the periplasmic pH elevation reported by Athmann *et al.* in confocal microscopy studies (41).

Methods

***Helicobacter pylori* culture.** Bacteria from frozen glycerol stocks (-80°C) were first streaked onto Brucella agar, supplemented with 5% horse blood (BBL, Becton Dickinson Microbiology, Cockeysville, MD, USA) and then incubated at 37°C under microaerophilic conditions containing 5 - 12% CO₂ and 5 - 15% O₂ for 24 hours. After initial culture on blood agar plates, bacteria were then rinsed into a liquid culture media consisting of brain heart infusion and other nutrients, and subsequently cultured in liquid broth for an additional 24 – 48 hours under constant gentle agitation at 90 rpm. Bacteria were removed from culture for study during the exponential phase of growth. In all studies reported here *H. pylori* were grown to an optical density of 1.0 at 600nm.

Mucin sample preparation. In this work Porcine Gastric Mucin (PGM) was used as a model system for human gastric mucin. It is important to note the distinction between purified PGM used in this work, which has been shown to reproduce the rheological properties of native mucus (13), and commercially available PGM from Sigma-Aldrich. The latter is partially degraded by protease treatment during preparation, contains high volumes of impurities and a comparative study (42) has shown that it does not reproduce the rheological properties of native gastric mucus. Here, PGM was obtained from pig stomachs and purified by Sepharose CL-2B column chromatography and density gradient ultracentrifugation as described previously (21). Samples for rheology were prepared by to the appropriate pH using a 10X artificial gastric juice solution consisting of hydrochloric acid and urea and left to equilibrate for 48 hours prior to study. *H. pylori* bacteria (ATCC strain 43504) were added to the PGM at 10X concentration after washing by centrifugation at 3900 rpm for 5 minutes and then rinsed in normal saline.

Rheology Studies. Rheological data was obtained using a TA instruments AR-2000 stress controlled rheometer. The instrument inertia was first calculated using the inertia calibration built

into the AR instrument control software, and then recalibrated for the inertia of the parallel plate geometry. Typically 400 μL of sample was available for study and the gap height was set to sufficiently fill the area of the 40 mm plate, usually 200-300 μm . A stress sweep at constant circular frequency was performed first to establish the regime of linear viscoelastic response for that sample, i.e., the region in which the applied shear stress is linearly proportional to the strain, and the viscoelastic moduli are independent of shear stress. Having identified a stress within the linear response regime for each sample studied, this value of stress was used in subsequent tests including a frequency sweep, creep test (step stress), and flow test (steady shear).

Microscopy and Imaging. *H. pylori* were imaged on an Olympus IX-70 inverted microscope using 20X and 100X phase contrast objectives. A Qicam cooled monochrome CCD camera (Qimaging, Burnaby, British Columbia, CA) mounted on the microscope was used to obtain sequences of digital images in the multi-paged TIFF format. Image sequences were then analyzed in IDL software to identify individual bacteria coordinates and link these together frame by frame to form trajectories using multiple particle tracking methods previously described by others (43, 44).

Two-photon Fluorescence Microscopy. Two-photon fluorescence images presented in this study were imaged using 800 nm excitation light from a pulsed laser source at a power of 100 mW. This illumination produced an excitation volume with full width at half maximum of 0.3 μm in the radial direction and 0.9 μm in the axial direction. Images were obtained with a scan rate of 5 kHz.

Figure Captions.

Figure 1: (A) Frequency dependent viscoelastic moduli for two PGM samples initially prepared to pH 4, one incubated with *H. pylori* and the other an identically prepared control sample. (B) Viscoelastic moduli plotted against applied oscillatory shear stress for *H. pylori* infected sample and control. (C) Steady shear data plotted as viscosity versus shear rate for sample with *H. pylori* and control sample.

Figure 2: Phase contrast images of *H. pylori* in gel phase PGM (upper left) and in solution phase PGM (upper right) with representative trajectories as obtained by particle tracking shown below (lower left and lower right respectively). At pH 2 *H. pylori* are confined in the mucin gel and exhibit no translational motility (note the dramatically smaller scale on the axes), whereas in the pH 6 mucin solution bacteria move about the field of view in large arcing spiral trajectories.

Figure 3: Snapshots from a time resolved sequence of 100X phase contrast microscopy images of a single *H. pylori* bacterium embedded in PGM gel illustrating flagella rotation from frame to frame. Consecutive images from left to right are resolved with 70ms time resolution.

Figure 4: (A) Displacement perpendicular to the long axis of the cell body as a function of time for a single bacterium trapped in mucin gel determined by tracking the centroid of the cell body in consecutive frames. (B) Frequency of oscillations for the same same bacterium calculated from cell body displacements above, and also determined by manually counting flagella rotation cycles.

Figure 5: *H. pylori* in PGM with 5mM urea initially driven to pH 2 by HCl immediately after sample preparation (A), at 5 minutes following preparation (B), and at 50 minutes following preparation (C). A plot of the corresponding rise in BCECF fluorescence intensity over time is shown in (D).

Figure 6: A schematic of the proposed mechanism by which *H. pylori* is able to attain motility in the mucus gel by triggering a sol-gel transition in the mucin glycoprotein component of the mucus.

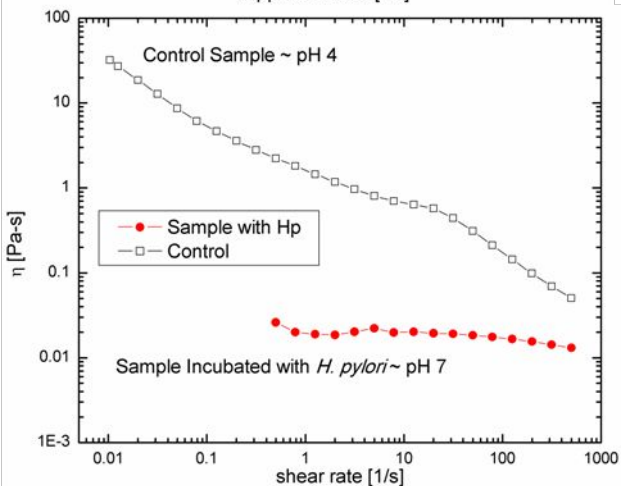
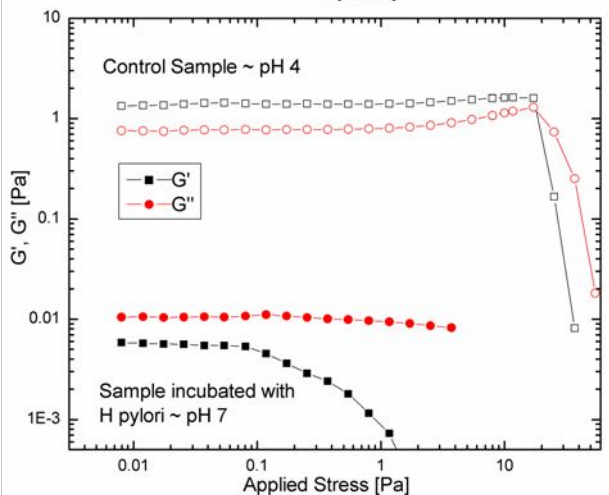
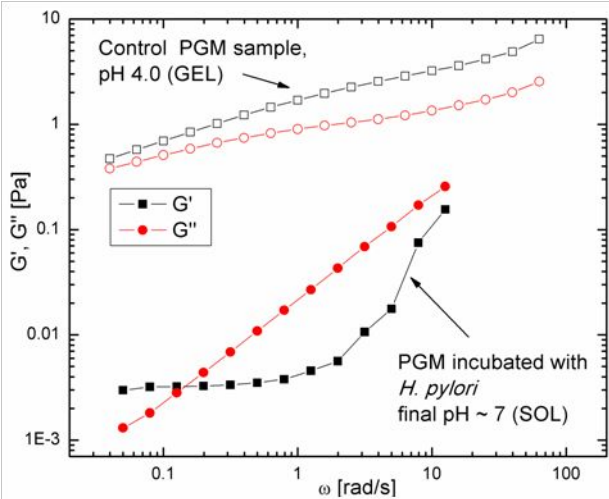
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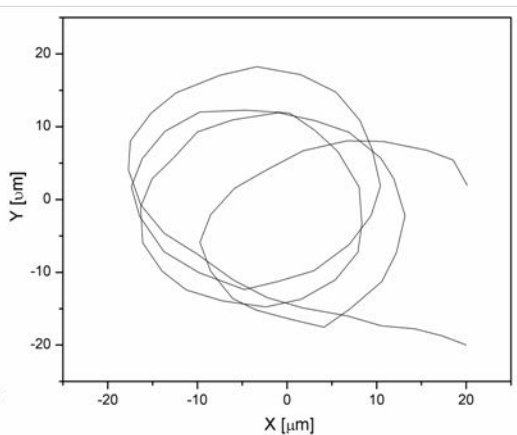
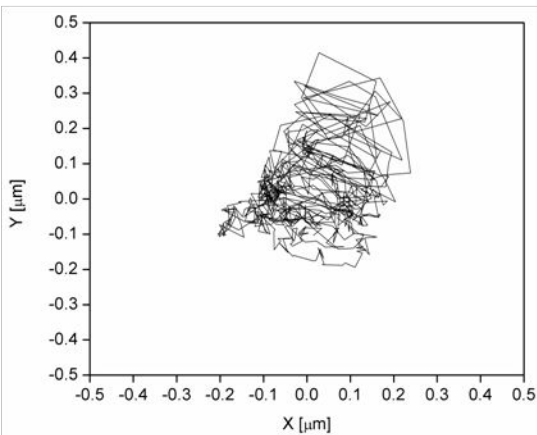
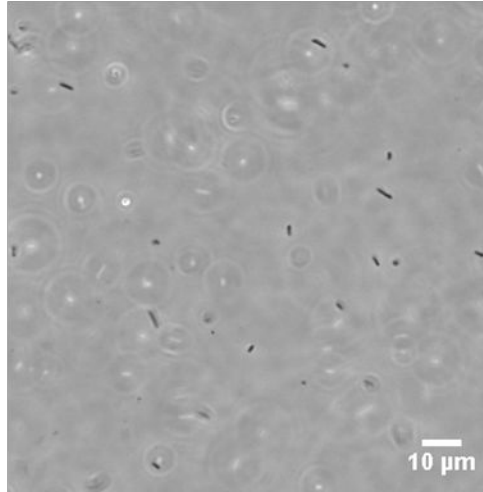
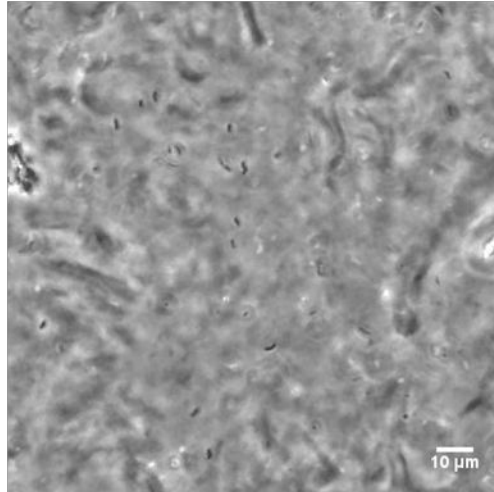
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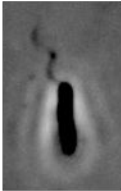
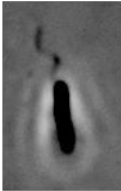
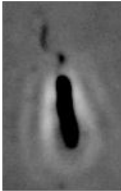
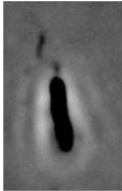
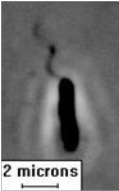
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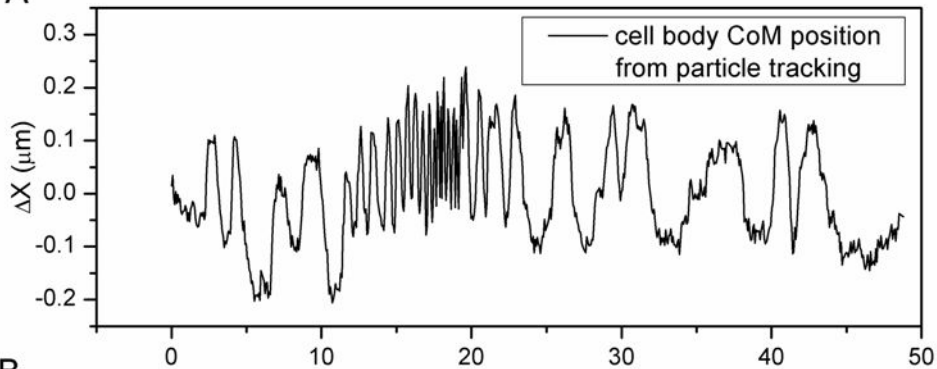
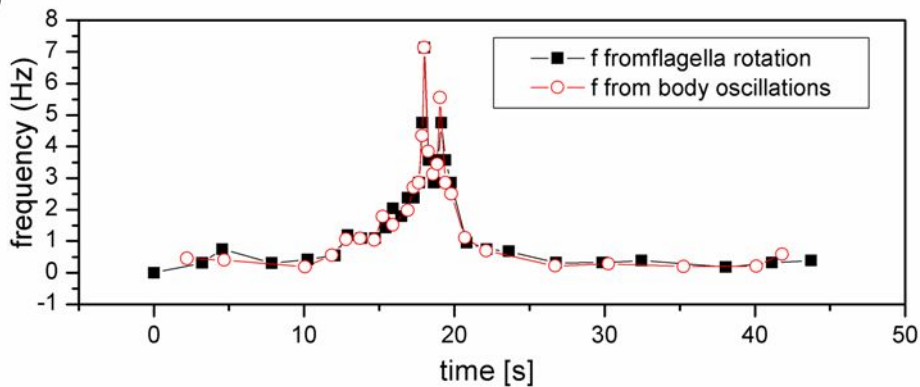
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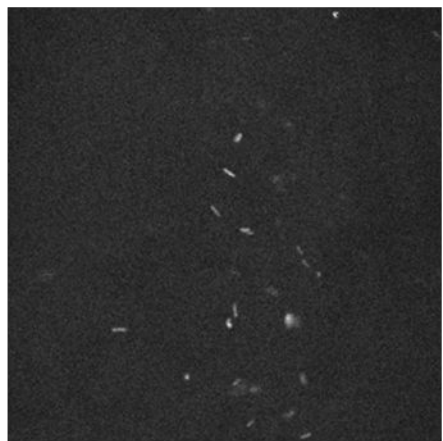
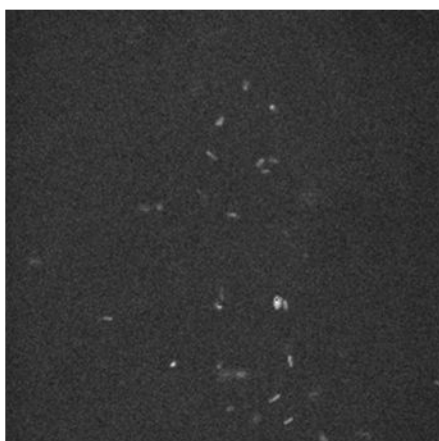
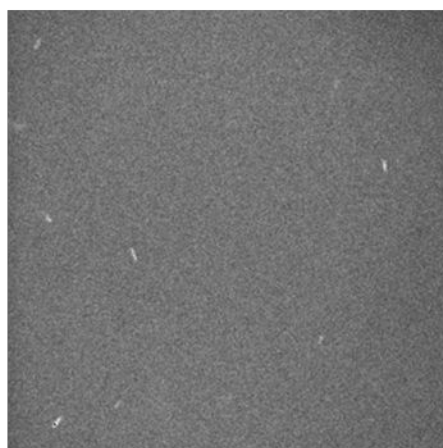
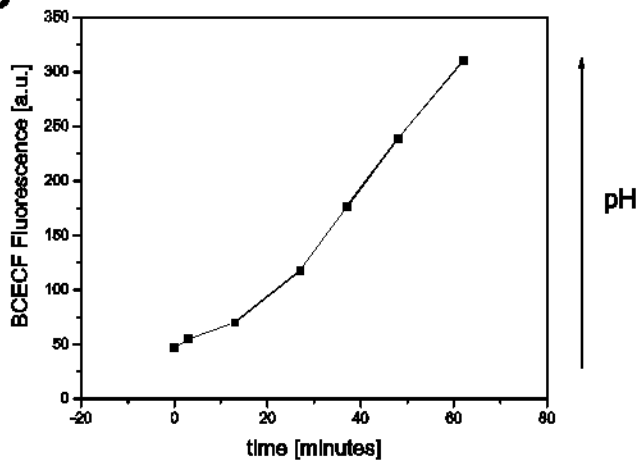
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A**B**

A**B****C****D**

HCl in stomach
lowers pH.

Gastric mucin
forms gel.

H. pylori urease causes:



HCl neutralized, pH \uparrow

Mucin
transitions
from gel to
sol.

Elasticity \downarrow

H. pylori swims
freely through
mucus and attaches
to epithelial cells