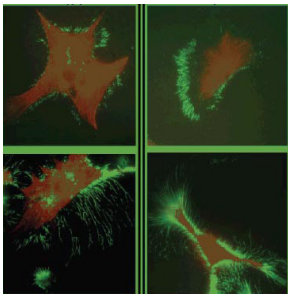

Hostos Journal of Student Research

A Publication of the Natural Sciences Department, Hostos Community College, CUNY

Spring 2011 Volume 3, Issue 1

In this issue:

Dear Student,

In this issue of the journal we offer you three research articles, one essay, and one laboratory report authord by your fellow students of the Hostos Community College.

We hope that you will enjoy all of these articles, and follow these authors in sharing your educational experience with Hostos community on the pages of the *Hostos Journal of Students Research*.

Editorial Board

Discovery of the DNA structure

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In April 1953, the scientific journal *Nature* published a short article that began with one of the greatest understatements in the annals of science. "We wish to suggest a structure for the salt of deoxyribose nucleic acid (DNA)" wrote the paper's authors, James Watson and Francis Crick (Fig. 1) . "This structure has novel features which are of considerable biological interest." (7). As the article went on to describe, the DNA molecule forms a "double helix" of two complementary nucleotide chains, where "nucleotide" refers to a molecular unit composed of a phosphate group (located on the outside of the helix), a five-carbon sugar (deoxyribose), and, in the interior of the helix, one of four nitrogenous bases (adenine, thymine, guanine, and cytosine).

Figure 1

Watson and Crick with their model of DNA

Today, the discovery of the structure of DNA ranks among the pivotal scientific breakthroughs of the twentieth century—on par with Einstein's special and general theories of relativity, the development of quantum physics, the invention of the digital computer, and the discovery of penicillin. Watson and Crick's paper described the biochemical mechanism that drives Mendelian or classical genetics, thus revolutionizing the study of biology and paving the way for modern biotechnology. (Prior to 1953, biochemistry and genetics were effectively, in Stephen Jay Gould's felicitous phrase, "nonoverlapping magisteria") (2). Little wonder, then, that a few hours after he and Watson hit on the correct structure, Francis Crick rushed into a Cambridge pub and proceeded "to tell everyone within hearing distance that we had found the secret of life." (7).

Watson and Crick's breakthrough was the culmination of several decades of intensive scientific research. In the ninety-odd years preceding the discovery of the structure of DNA, biologists had established many of the laws that govern the process of heredity. Much of this work was done in the 1860s by an Austrian priest named Gregor Mendel, who deduced his laws of heredity from experiments that he conducted with pea plants. Although Mendel's work fell into relative obscurity soon after it was published, it was rediscovered around the turn of the century. Not long after, in his 1915 book *Mechanism of Mendelian Heredity,* the American biologist Thomas Hunt Morgan proposed that the genes described by Mendel were arranged linearly on the cellular chromosomes (4).

During the same period, organic chemists had made great strides in the study of carbon and its compounds. DNA was first isolated and identified as coming from cell nuclei by the German chemist Johann Friedrich Miescher. (Although Miescher speculated that DNA, which he referred to as "nuclein," might play a role in the process of heredity, he believed that genetic diversity was too complex to be determined by a single molecule) (1). Later, in the two-and-a-half decades spanning the turn of the century, the German chemist Emil Fischer determined the composition and structure of a wide range of organic compounds, including many sugars, purines (a class that includes adenine and guanine), and amino acids (5). Then, in 1929, the Russian-American biochemist Phoebus Levene determined that nucleic acids contain deoxyribose (1). By the 1940s, the search for the biochemical mechanism of genetic inheritance was underway.

When James Watson first became seriously interested in genetics, while working on his Ph.D. in zoology, it was unclear whether the molecule responsible for storing genetic information was a nucleic acid or a protein. Some scientists argued that nucleic acids, with only four nucleotide bases, lacked the complexity to guide heredity; they believed that proteins, with twenty different amino acids,

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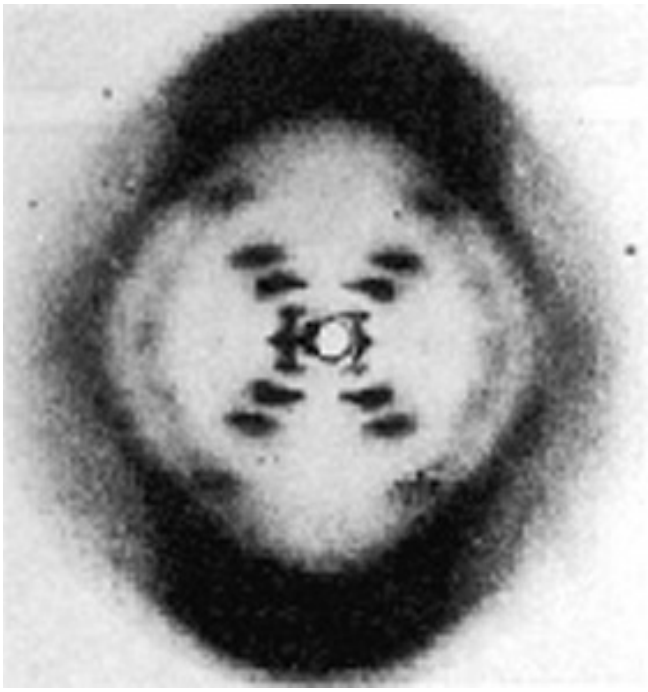
were better suited to the task. However, in 1944, the Canadian-born bacteriologist Oswald Avery provided strong experimental evidence that the carrier of genetic information was in fact DNA (8) . As Watson put it in *The Double Helix*, "Avery's experiment made [DNA] smell like the essential genetic material. So working out DNA's chemical structure might be the essential step in learning how genes duplicated. Nonetheless, in contrast to proteins, the solid chemical facts known about DNA were meager. Only a few chemists had worked with it and, except for the fact that nucleic acids were very large molecules built up from smaller building blocks, the nucleotides, there was almost nothing chemical that the geneticist could grasp at." (6).

One hint of the structure of the DNA molecule came from the work of the chemist Linus Pauling, who had recently proposed that polypeptide chains sometimes fold into a helical structure (technically known as an "alpha helix"). Although Pauling arrived at this insight from theoretical and aesthetic considerations, he was later proven correct. Pauling later proposed — mistakenly—that the DNA molecule was a triple helix composed of nitrogenous bases surrounding a sugar-phosphate backbone (5). Nonetheless, both the idea of a helical structure and Pauling's straightforward theoretical approach—"using tinker-toy-like models to solve biological structures", as Watson characterized it —had a profound influence on Crick and Watson. "We could thus see no reason why we should not solve DNA in the same way [as Pauling had solved the structure of alpha helix]," wrote Watson in *The Double Helix*. "All we had to do was to construct a set of molecular models and begin to play—with luck, the structure would be a helix" (6).

Another crucial clue came from the work of Maurice Wilkins and Rosalind Franklin, two chemists at King's College London who were using a technique known as X-ray crystallography to study the structure of DNA. In fact, it was an X-ray photograph taken by Franklin (and shown to Watson by Wilkins without Franklin's knowledge) that suggested to Watson and Crick that DNA formed a double helix with the phosphate-sugar backbone on the outside and the bases on the inside (Fig. 2) (6).

The final pointer came from the Austrian-born biochemist Erwin Chargaff, who in 1950 analyzed the relative proportion of each nitrogenous base in DNA and discovered not only that the amount of adenine was equal to the amount thymine, but also that the amount of guanine was equal to the amount of cytosine. (2) but also that the amount of guanine was equal to the amount of cytosine. (2). The fact that certain bases occurred in equal amounts suggested that they are paired together; more precisely, it suggested that adenine is paired with thymine, and that guanine is paired with thymine. Indeed, a close inspection of the molecular structure of these bases shows that they can be made to fit together perfectly in the interior of the double helix, with each pair held to its complement by hydrogen bonds. Moreover, as Watson explained in *The Double Helix*, the pairing of nitrogenous bases also provides a mechanism for the replication of the DNA molecule: "Always pairing adenine with thymine and guanine with cytosine meant that the base sequences of the two intertwined chains were complementary to each other. Given the base sequence of one chain, that of its partner was automatically determined. Conceptually, it was thus very easy to visualize how a single chain could be the template for the synthesis of a chain with the complementary sequence" (6).

Figure 2



Rosalind Franklin's X-ray crystallography photograph

Almost as soon as Watson and Crick presented the complementary-base-pair model to Wilkins and Franklin, the two King's College scientists recognized its elegance, its simplicity, and its explanatory power. The two were convinced that it must be the correct structure, and a quick review of their experimental data provided additional confirmation that it was. All four scientists published their findings in the same 1953 issue of *Nature*, and followed up with additional papers in the succeeding months.

As a result their work, James Watson, Francis Crick, and Maurice Wilkins jointly received the 1962 Nobel Prize in physiology or medicine. Unfortunately, because she had died of ovarian cancer in 1958, Rosalind Franklin did not receive a Nobel. (The Nobel Prize bylaws stipulate that all prize recipients must be living at the time the prize is given).

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Finding Parabolic Profiles in Projectile Motion: Proving Galileo Galilei's Work.

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Since the scientific revolution many scientist studied the laws that govern physical phenomena. During this period physicist Galileo Galilei revolutionized the world with his theories and discoveries on free fall and projectile motions. This latter motion has a very important aspect that is useful when analyzing it. Two dimensional motions can be resolved into two independent motions; one along the horizontal direction, and the other one along the vertical direction. By analyzing these two directions, which are the two components of the trajectory, Galileo, as well as we, were able to conclude that the path of this motion is a smooth curve or a semi-parabola. By using our experimental design we were able to support this discovery. In our model we first measured the horizontal displacement a steel ball took at different heights. We then measured the initial velocity, acceleration, and gravity of the system by using the kinematic equations. After obtaining our data we graphed the horizontal displacement with respect to each height. We then analyzed our data and discovered that a parabola best fit our data by approximately 99 percent.

Introduction

Projectile motion is a special case of a two dimensional motion with a constant acceleration (1). The path the object makes was first described by the scientist Aristotle. His theory says that after the object is given an initial force "impetus", as it was called, the object moves in a straight line up to a point, and then falls straight to the ground (2). With more careful observation, Galileo Galilee realized that objects do not move as Aristotle thought; but rather in a smooth curve (2). One of Galilee's hypotheses was that the motion of a ball given an initial velocity will take the shape of a parabola (2).

The main purpose of this experiment is to demonstrate that this motion is actually a parabola. This experiment was divided in two different parts; first the horizontal displacement of the ball after leaving the inclined plane at a fixed height was measured. Second, the initial velocity, acceleration, and the gravitational force were determined.

Experimental Design

Apparatus:

- Inclined Plane.
- Plastic Pipe
- Steel Ball
- Stand.
- Two clamps.
- Meter stick
- Photo gate.
- Carbon Paper.
- Tape.
- Paper and pen.
- Chalk or Bright colored tape.

Procedure:
Part A: *Determining Horizontal Displacement of Projectile Motion.*

To begin the experiment, set up the incline plane system. Select the angle α between 0^0 and 90^0. Make sure the incline plane system is on the edge of the table. Select the first height and then adjust the stand to that height. Place two sheets of carbon paper on the stand, use tape to mount the paper to the stand. Place the steel ball on the top of the incline plane system. Release the ball and record the horizontal distance it has traveled from the end of the incline to its final position marked by the carbon paper. Repeat at least 50 trials of the horizontal

displacement. Repeat this experiment and record the horizontal distance for five different heights.

Part B: *Determining the Initial Velocity of the Projectile Motion.*
Install a photo gate system to the incline plane. Place one sensor on each end of the incline plane. Measure the distance between the two sensors; this will be the displacement used when calculating velocity. Be sure to place the top photo gate sensor 2 cm away from the entrance of the plastic pipe. Release the ball and record the time it takes to reach the bottom sensor. Repeat this process and have at least 100 trials, if any of the measurements do not agree with 5% repeat the trial. Calculate the average time for all of your trials. Using the average time and displacement, determine initial velocity, acceleration on the inclined plane, and acceleration due to gravity of the steel ball.

Results

Part A: Determining Horizontal displacement of projectile motion.

For the first part of the experiment the angle used was 15°. This remained constant throughout the experiment. The objective throughout the first part of the experiment was to determine the horizontal displacement of the steel ball at different heights. For each height a minimum of 50 trials were conducted. Table 1 shows the measurements obtained at each height.

After obtaining the horizontal displacement for each height, we graphed the motion to test Galileo Galileo's work (Figure 1); according to him and our hypothesis, the balls motion should be in parabola profile. According to R. H. Naylor, in order to find the shape of the trajectory in a projectile motion, Galileo Galilee started from two basics features, which are inertia and the law of free fall (3). Because the gravity pulls downwards an object to the earth, and the projectile moves forward horizontally at uniform motion, and time. This trajectory is mathematically called parabola. That shape is clearly showed in Figure 1 as the same way that Galileo did in his work of projectile motion.

Figure 1
Displacement on the X-direction vs. Y-direction

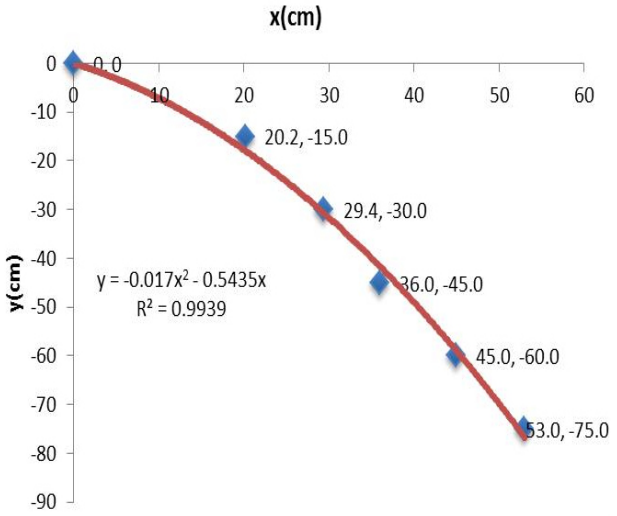
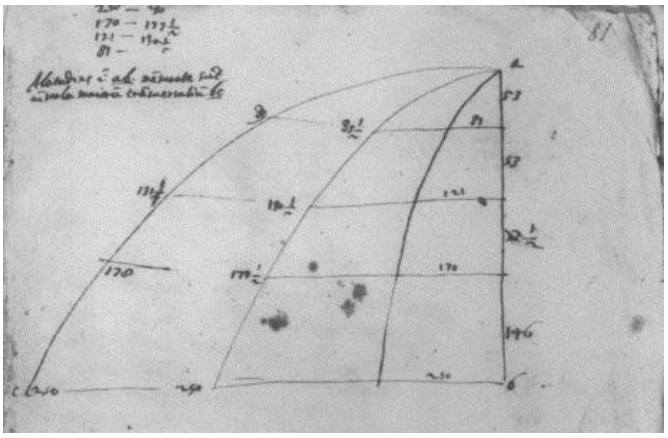


Table 1
Measurements of horizontal displacement during the projectile motion

Height	Average Height (from the table to the floor) (cm)	Average Height(m)	Average Length (where the ball hits the ground) (cm)	Average Horizontal Displacement (m)	Error of Measurement (mm)
H_0	0	0	0		
H_1	-15.0	0.150	20.2	0.203	0.001
H_2	-30.0	0.300	29.4	0.244	0.001
H_3	-45.0	0.450	36.0	0.360	0.001
H_4	-60.0	0.600	45.0	0.450	0.001
H_5	-75.0	0.750	53.0	0.530	0.001

Figure 2
Galileo's Galilei's Experiment (4).



Part B: *Determining initial velocity of the projectile motion.*

For the second part of the experiment the objective was to measure the steel balls displacement and determine its initial velocity, acceleration on the inclined plane, and acceleration due to gravity. Measurement of displacement and time was the first step in obtaining the rest of the physical quantities (Table 2). Figure 2 depicts the distribution of the time measured in a histogram and Table 3 lists the formulas used to calculate velocity, acceleration, and gravity.

Table 2
Times measured with photo gate

Time (ms) trials			
756	796	778	777
773	800	785	784
788	791	790	776
763	778	784	786
773	795	780	789
773	790	791	778
776	785	790	786
767	774	781	782
771	780	777	782
763	789	784	794
761	781	795	777

Figure 3
Histogram of times measured by photo gate

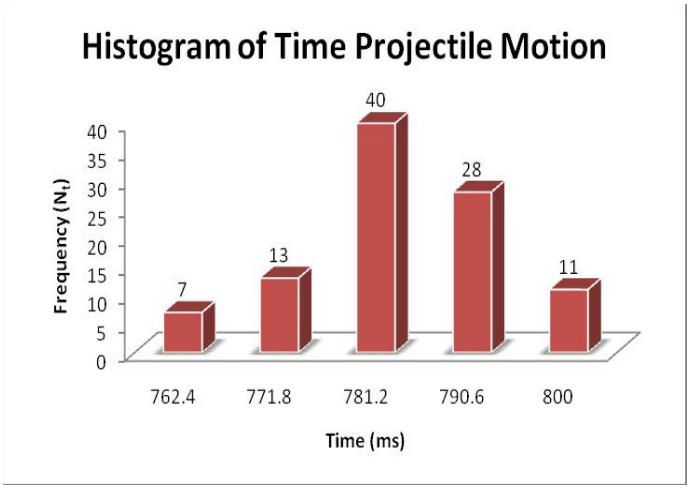


Table 3
Values for velocity, acceleration, and acceleration due to gravity

Calculation	
Velocity (v) (m/s)	1.470±0.004
Acceleration (a) (m/s^2)	1.880±0.008
Acceleration due to Gravity (g_1) (m/s^2)	10.16(4%)*
Acceleration due to Gravity (g_2) (m/s^2)	7.26(26%)*
Data of Inclined Plane	
Displacement (Δx) (m)	0.570±0.001
Average Time (ms)	778±1

*This represents the percentage error calculated with respect to the standard value (g = 9.80 m/s^2).

The calculations shown in Table 3 were obtained by using the kinematic equations for constant acceleration listed in Table 4. We compared our experimental values for acceleration due to gravity with the standard value (g = 9.80 m/s^2)(5).

In our calculations we considered two assumptions, first the ball was only translating (i.e. a = g sinα), and second the ball was translating and rolling without slipping, which takes into account the moment of inertia of the ball (Table 4).

Table 4
Constant acceleration formulas (5).

Constant Acceleration Formulas	
Solving for velocity (v) in this equation, $v^2 = v_0^2 + 2a(\Delta x)$ where initial velocity is 0 ($v_0 = 0$) and Δx represents displacement Velocity is $v = \sqrt{2a\Delta x}$	Solving for acceleration (a) in this equation, $\Delta x = v_0 t + \frac{1}{2}at^2$ where initial velocity is 0 ($v_0 = 0$) and Δx represents displacement Acceleration is $a = 2\Delta x / t^2$ *Also,* $a = g \sin\alpha$ *(Considering only translational motion) and* $a = \frac{5}{7} g \sin\alpha$ *(Considering Translational and Rotational Motions)*
Substituting acceleration (a) in this equation, $v = \sqrt{2a(s)}$, and solving for velocity (v) we obtained $v = \sqrt{2\left(\frac{2\Delta x}{t^2}\right)\Delta x} = 2\Delta x / t$ Error Propagation for velocity and acceleration quantities: $\Delta v = \frac{2}{t_{average}}\left[\Delta(\Delta x) + (\frac{\Delta x}{t_{average}})\Delta t\right]$ $\quad$ $\Delta a = \frac{2}{t^2_{average}}\left[\Delta(\Delta x) + (\frac{2\Delta x}{t_{average}})\Delta t\right]$ Where $\Delta(\Delta x)$ and Δt represent the absolute uncertainty for the displacement and time, respectively.	

Table 5
Parabolic equation obtained for standard projectile motion

Equation obtained of the Parabola Graph	
X-Component $v_{0x} = v_0 \cos\alpha$ Constant acceleration formulas: $\Delta x = v_{0x} t + \frac{1}{2}at^2$ Where acceleration is 0 ($a = 0$), and $v_{0x} = v_0 \cos\alpha$ $\Delta x = (v_0 \cos\alpha)t$ Solving for time (t): $t = \Delta x / (v_0 \cos\alpha)$	Y-Component $v_{0y} = v_0 \sin\alpha$ Projectile motion equation $\Delta y = v_{0y} t + \frac{1}{2}gt^2$ Where $v_{0y} = v_0 \sin\alpha$, $\Delta y = (v_0 \sin\alpha)t + \frac{1}{2}gt^2$
Substituting time (t) in this equation $\Delta y = (v_0 \sin\alpha)\, t + (\frac{1}{2})\, gt^2$, and solving for (y) we obtained the equation below. $y = -\frac{1}{2}\left(\frac{g}{v_0^2(\cos\alpha)^2}\right)x^2 - (\tan\alpha)x$	

Discussion and Conclusion

The objective for the first part of the experiment was to determine the horizontal displacement of the projectile motion. As Table 1 illustrates, as the height from the table decreased the horizontal displacement of the projectile motion increased. After several trials the steel ball began to land on one specific position. This pattern was noticed at all the different heights, while the angle remained 15°. After completing all the trials, the measurements were then graphed.

As the linear regression in Figure 1 shows, the balls trajectory has the same properties as that of a parabola. Our correlation coefficient (R^2) for the graph was 0.9939; this meant that the parabola curve fits our data by about 99%. For the second part of the experiment the objective was to determine the initial velocity of the projectile, acceleration, and gravity. Using the displacement measured and the average time, we obtained a velocity of 1.47 m/s.

In determining the value for gravity we considered two cases. The first case the ball was only translating, and the second case was the ball was rolling without slipping. In the first case we did not consider the moment of inertial of the ball. The value obtained during the first case was 7.26m/s2, compared to the standard g value, this value contained 26% error. When we calculated acceleration due to gravity the second time the value obtained was 10.16 m/s2 which only contained 4% error. A possible factor that may have contributed to the small error is air resistance. This force was neglected during the calculations. After evaluating our data we have come to the conclusion that Galileo's original hypothesis was correct. Our data supported our hypothesis that projectile motions are modeled after parabolic functions. We also proved that projectile motion is dependant of the initial velocity of the system.

Acknowledgements

We would like give special thanks to our physics professor Yoel Rodríguez for his help and advice throughout our project. We also want to thank our lab technician Jaime Luján for providing us with the correct guidance and supervision, without their support completing this study would have been difficult.

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Effect of Acidic pH on Expression of Heat-Labile Enterotoxin (LT) by Escherichia coli (E. coli).

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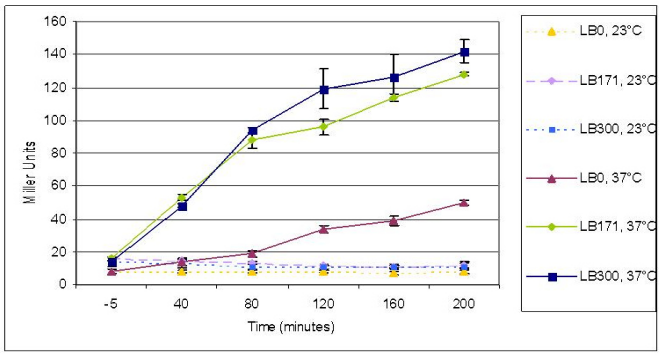
Escherichia coli (E. coli) is one of the many types of bacteria that can be found in the intestines of human beings and animals. However E. coli can also be pathogenic such as enterotoxigenic strains of E. coli (ETEC) and is one of the leading causes of diarrhea in human beings especially in children under the age of five. ETEC strains carry transmissible Ent plasmids, which encode heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST), or both. E. coli is transmitted by the fecal-oral route. Thus, E. coli naturally inhabit environments that can differ markedly in pH, temperature, osmolarity, and nutrient availability. LT expression has been shown to be affected by environmental conditions such as temperature and osmolarity. It has been determined that the regulatory protein, histone-like nucleoid-structuring protein (H-NS) is involved in the temperature regulation of LT. The effect of acidic pH on LT expression is less well characterized. This research focuses on the effects of acidic pH in an E. coli strain carrying a plasmid where the LT genes are fused to the E. coli's beta galactosidase gene. This plasmid has all of the regulatory regions present. Beta galactosidase assays were performed to measure the amount of LT - beta galactosidase fusion protein from E. coli grown under different pH conditions ranging from pH 5.5 to neutral pH. We found that growing the E. coli at acidic pH inhibited their ability to produce the LT- beta galactosidase fusion protein. This is consistent with the fact that ETEC strains bind to the ileum and jejunum of the small intestines, where the pH is close to neutral. This knowledge will help us to identify further the transcriptional factors that regulate the expression of LT.

Introduction

Enterotoxigenic strains of *Escherichia coli* (*E. coli*) (ETEC) are a leading cause of infectious diarrhea in human beings and animals in developing countries (1). ETEC is also a common source of traveler's diarrhea, in endemic countries such as Mexico and Bangladesh. ETEC is frequently encountered by tourists, members of the military or other visitors (2). Close to 500,000 deaths in children due to ETEC are expected annually (3). ETEC colonizes the small intestine and produces plasmid-encoded heat-labile (LT) or heat-stable (ST) enterotoxins or both (4). LT is similar in molecular size, sequence and function to cholera toxin (CT) produced by *Vibrio cholerae* (5). LT has six subunits. It consists of five identical B subunit (LTB) and a single A unit (LTA). The A subunit has two domains linked by a disulfide bridge (A1, the active toxin portion of molecule, and A2 is the helical portion of the molecule that gives the B subunit anchorage (6). The B subunits bind to receptors on the small intestine while the A subunit enters into the intestinal cell (1). *eltA* and *eltB* genes (previously called *etxA* and *etxB* or *toxA* and *toxB* (7) in earlier studies) form a two-gene operon (5, 8). *E. coli* naturally inhabit environments that can differ markedly in pH, temperature, osmolarity, and nutrient availability (1). Studies show that ETEC produces more LT at 37˚C as compared to lower temperatures (1,9). In addition, oxygen levels and osmolarity were found to influence toxin expression. Specifically, microaerophilic conditions and increased salt concentrations both promote the production of LT (9). A regulatory protein, histone-like nucleoid structuring protein (H-NS) has been determined to be involved in the temperature regulations of LT (1). Recent findings show that the upstream control region (UCR) located upstream of the LT promoter is not involved in LT temperature regulation; however, there is a region downstream of the promoter in the coding region of the *elt*A involved in LT temperature regulation. This region is referred to as the downstream regulatory element (DRE) (1). Deletion of DRE in the *elt*A gene elevates the levels of mRNA transcribed from the operon's promoter. As mentioned earlier, salt concentrations and oxygen levels also influence LT expression. LT expression is elevated about 4-5 fold when cells are grown microaerophilically in LB medium containing 0.171 M NaCl or LB with 0.3 M NaCl as compared to LB medium without any NaCl additions. Temperature has the most dominant effect on LT expression but it can be further influenced by NaCl levels (see Figure 1). However, in regard to the regulatory effects mediated by NaCl on LT expression, H-NS does not seem to be involved (9).

Figure 1: Effect of temperature and osmotic upshift on LT expression.



Continuous lines represent cultures grown at 37°C and dashed line represent cultures grown at 23°C. Symbols: filled triangle, 0 M NaCl; filled diamond, 0.171 M NaCl; cross, 0.3 M NaCl. Values are the mean _ SEM of triplicate determinations in three independent experiments. (*Permission for use from author of ref. 9*).

Prior to this study, it had been unclear exactly what the effect of acidic pH on LT expression was. In this study, the effects of acidic pH on the expression of the heat-labile enterotoxin produced by a strain of E. coli containing a full-length LT-beta galactosidase fusion plasmid was evaluated. Among the ways this was done was by assessing how acidic pH effects LT expression when cells were grown initially at 25˚C microaerophilically at pH 5.5 and then temperature upshifted to 37˚C and grown either at pH 5.5 or pH 7.2 (with or without buffer). The data presented here support the hypothesis that growing the *E. coli* at acidic pH inhibited their ability to produce the LT-beta galactosidase fusion protein.

Materials and Methods

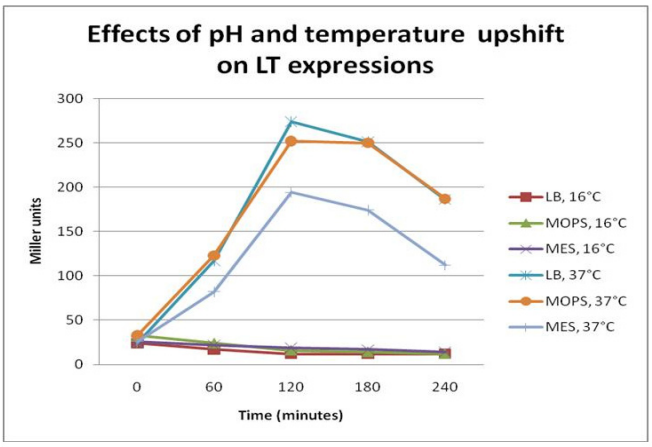
Bacterial strains, plasmids and growth conditions: X7026 (lac$^-$ *E. coli* strain), which has been largely used for LT beta galactosidase studies, was obtained from Dr. J.D. Trachman, Natural Science Department, Hostos Community College and was used for this study. Luria-Bertani (LB) broth medium was prepared with 10 grams of tryptone per liter and 5 grams of yeast extract per liter. Also 10g of NaCl per liter was added to the LB medium. 3-[N-Morpholino] propanesulfonic acid (MOPS, pKa 7.2) and 2-[N-Morpholino] ethanesulfonic acid (MES, pKa 6.1) was also used to buffer LB media. Both MOPS and MES were used at 100mM. The pH of the buffered media was then adjusted to 5.5 in the case of LB-MES and 7.2 in the case of LB-MOPS, by using sodium hydroxide (NaOH). Bacteria were grown in tightly capped culture tubes (13 mm), which were filled to one-third, to one-half capacity. X7026 were transformed using a modification of the procedure described at http://www.biologyjunction.com/sample_4_lab_6a_transformation.htm. Four colonies of freshly grown *E. coli* from a Tryptic Soy Agar (TSA) plate were inoculated into 1 ml of 0.05M $CaCl_2$ solution (equivalent to one colony per 250 μl of the solution) for 3 hours on ice. The bacterial suspension was then divided into four sterile 1.5 ml microcentrifuge tubes. Some aliquots had plasmid DNA added to the tubes and one tube (negative control) had none. The tubes were incubated for 45 minutes on ice. Then later, the tubes were heat-shocked at 42°C for 90 seconds and then placed on ice again for about 1 minute. A small volume (250 μl) of warm Tryptic Soy Broth was added to each of the tubes and the tubes were incubated for 30 minutes at 37°C. Aliquots of the bacteria were then plated on pre-warmed TSA containing 30 μg/ml of ampicillin. X7026 was transformed with one of the three plasmids [a full-length LT-beta galactosidase fusion plasmid (pJT8) or one of two plasmids where portions of the UCR have been deleted (p1-80 where approximately 250 base pairs remain upstream of the native LT promoter or p6F-12, where all of the base pairs upstream of the native LT promoter have been deleted)]. Overnight (ON) X7026 cultures containing the pJT8 LT-beta galactosidase plasmid were grown in LB-MES medium, pH 5.5, containing 0.171M NaCl at 16°C under microaerophilic conditions. The next day approximately 30 μl of each ON culture was transferred to fresh LB medium of different pHs and allowed to continue growing at 16°C under microaerophilic conditions until early log phase. The resulting cultures were then incubated at either 16°C or 37°C microaerophilically. Periodically, the culture absorbances at 580 nm were measured, the time recorded and aliquots were taken out and stored at 4°C until they were assayed for beta galactosidase activity. Culture absorbances at 580 nm were obtained using a Spectronic 20 spectrophotometer. Beta galactosidase assays were performed as follows. In this study, from each subculture tube 100 μl well mixed aliquots were placed in borosilicate cuvettes (13mm x 100 mm). Z buffer was added to the tubes containing the bacteria to bring the final volume to 1.9 ml and a control tube containing 1.9 ml of Z buffer was also prepared. The Z buffer consisted of 16.1g/L of $Na_2HPO_4x7H_2O$, 5.5g/L of NaH_2PO_4 xH_2O, 0.75g/L of KCl and 0.246g/L of $MgSO_4x$ $7H_2O$. Z buffer was supplemented with 2.7ml/L of β-mercaptoethanol at the time of the assay.

Next, 80 μl of chloroform ($CHCl_3$) and 40 μl of 0.1% sodium dodecyl sulfate (SDS) were added to permeabilize the bacteria. All the tubes were vortexed for 10 seconds and 400 μl of the freshly prepared substrate O-nitrophenyl--D-galactopyranoside (ONPG) [4 mg/ml] was added. Once the desired yellow color appeared, 900 μl of 1 M sodium carbonate (106 g/L) was added to stop the reaction. The time was then recorded, and the absorbance reading for beta galactosidase activity was obtained at 550 nm and 420 nm using a Spectronic 20 (Bausch and Lomb). Minimally, each sample was tested in triplicate. Data are expressed as mean based on the values obtained. The enzymatic activity is expressed in Miller units. The equation is as follows:

Enzyme Activity (Miller Units) =
1000 X [O.D. 420 - (O.D. 550 X 1.75)]
(Time)(O.D.subculture)(Volume of the culture added to reaction)

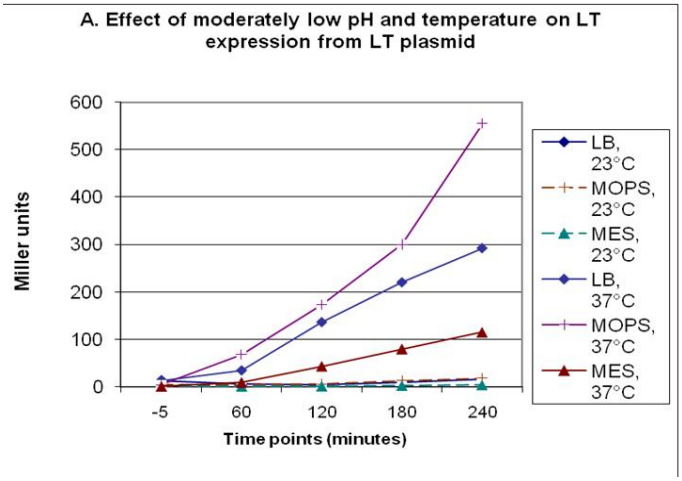
Results

Figure 2
Effect of pH and temparature upshift on LT expression.



The graph above shows that, at a lower pH (5.5) and lower temperature (LB 16ºC, MES 16ºC and MOPS 16ºC), the amount of LT expressed was low. As expected, the amount of LT expressed by the bacteria was higher in the cultures grown at 37°C, regardless of the pH of the growth medium. Bacteria grown in LB at pH (7.2) with or without MOPS buffer and at high temperature (37ºC), expressed approximately 1.7 times more LT than the bacteria grown in LB buffered with MES (pH 5.5) at 37ºC. Surprisingly, as cultures grown at high temperature (37°C) reached 240 minutes, there was a decline in the amount of LT expressed. Comparing the results presented in Fgure 2 to the results presented in Figure 3 (unpublished data, Trachman), the decline in the amount of LT expressions at 240 minutes by all of the 37°C cultures did not correspond to earlier results. These results showed that LT expression continued to increase as the cultures were further incubated between 180 minutes to 240 minutes. Also, the preliminary results showed a more substantial increase in LT expression between the cells grown at 37°C and pH 7.2 as compared to those grown at 37°C and pH 5.5 and there was also a significant difference between bacteria grown at 37°C and pH7.2 with MOPS buffer (5.5-fold effect when compared to cells grown at 37°C and pH 5.5) or grown at 37°C and pH7.2 without MOPS buffer (3-fold effect when compared to cells grown at 37°C and pH 5.5). It is not clear why the LT expressions declined as the time reached 240 minutes, or why there was less of a difference in the cells grown at 37°C at the different pH conditions. There could have been some bacterial contamination with the media or some technical errors while pipetting the bacteria for the 240 minute time points. Also, for the previous results obtained by Trachman (unpublished data), overnight cultures were grown for 30 hours and in doing these experiment, the subcultures were grown to an OD value of 0.10 before starting the temperature upshift part of the experiment as compared to our growing the overnight culture for 17 hours and growing the subcultures to an OD value of 0.05 before starting the temperature upshift part of the experiment. The temperature in the laboratory was also considerably below typical room temperature (the room was at 16°C rather than 22ºC). Regardless, these are the results we obtained. The experiments will be repeated to gain more knowledge on why the LT expression should either decline or continue to rise at 240 minutes and to determine what the strength of the effect of acidic pH is on LT expression.

Figure 3.
Effect of moderately low pH and temperature on LT expression from LT plasmid.



Discussion

LT expression for X7026 carrying the full-length plasmid pJT-8 was low at low pH (pH 5.5) and high at relatively neutral pH (7.2).This is consistent with the fact that, ETEC strains binds to the ileum and jejunum of the small intestine, where the pH is close to neutral. In the future, the experiment will be repeated to verify the decline or continuous rise in LT expressions at 240 minutes, also X7026 carrying the smaller plasmids where portions of the UCR have been deleted (p1-80 where approximately 250 base pairs remain upstream of the native LT promoter or p6F-12, where all of the base pairs upstream of the native LT promoter have deleted) will be tested.

Acknowledgements
We would like to thank Ms. Chew Yap for her assistance in carrying out these experiments, and the LSAMP program for their financial support of Mr. Jacob Taye.

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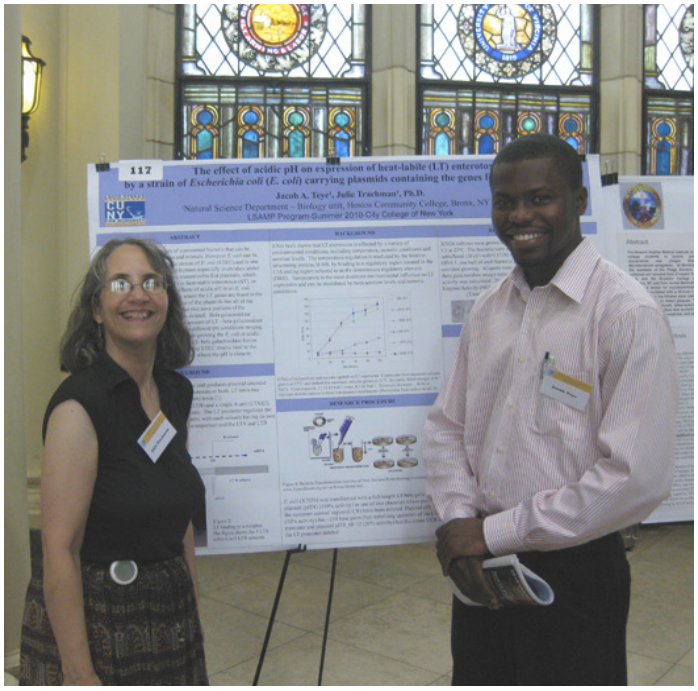

Professor Julie Trachman and Mr. Jacob Taye present results of their research at the summer NYC-LSAMP conference at the City College, CUNY, July 29, 2010.

Identification and Isolation of brh2/est2 Double Mutant in Ustilago maydis.

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A mutation in BRCA2, a tumor suppressor gene, was found in many types of cancer cells in humans (4). One of the events of cancer progression is the development of its cells' capacity to multiply indefinitely, a process called immortalization (2). During immortalization cells acquire an ability to excessively elongate the end regions of their chromosomes called telomeres (2). One of the pathways leading to telomere elongation is an activation of the telomerase, which is a nucleoprotein complex required for telomere extension (2). It was proposed that the inactivation of telomerase in tumor cells will inhibit immortalization and thus prevent the cancer transformation. The goal of this project was to create a model system that imitates cancer cells with mutation in the BRCA2 gene and inactive telomerase. We used a fungal plant parasite Ustilago maydis as our model organism. U. maydis carries both the homolog of BRCA2 gene called BRH2 and EST2 gene, which is a catalytic subunit of telomerase (3).

Introduction

BRH2 is a Ustilago homolog of a human tumor suppressor gene *BRCA2* (Breast cancer). It functions in the DNA recombination-repair pathway and may also be involved in the regulation of cell division (2, 4). Loss of *BRAC2* function leads to inability of a cell to repair mutations and is followed by accumulation of DNA defects and development of cancer (4). In 2002, Dr. William Holloman, Dr. Milorad Kojic and their team of researchers discovered the homolog of the *BRAC2* gene in *Ustilago maydis*—and named it *BRH2*. Just like *BRAC2* gene, *BRH2* plays a role in DNA repair, recombination, and genomic stability (3) . Loss of *BRH2* in *Ustilago* leads to an extreme sensitivity of its cells to DNA damaging agents, such as UV radiation (3).

Telomeres are specialized nucleoprotein structures located at the end of chromosomes (2). Though they do not code for any genomic information, telomeres are important in maintaining genome stability. Due to the nature of DNA polymerase, the protein responsible for the DNA replication, telomere length decreases in each round of DNA replication (2). When telomere lengths become critically short, cells senesce and die. To be able to replicate indefinetely, cells require to develop an ability to maintain or significantly increase telomere length. This can occur through two pathways: increase in telomeric DNA length by the action of specialized enzyme telomerase, or through the process of homologous recombination (2).

Telomerase is a ribonucleoprotein complex located at telomeres. The telomerase complex possesses a reverse transcriptase activity capable of adding nucleotides to the 3' end of linear single-stranded DNA, thus replenishing the telomere loss normally incurred during each replication cycle. In somatic cells, telomerase is normally either not expressed at all or expressed at very low level. During tumor immortalization, telomerase expression is activated, which leads to excessive telomere elongation (2).

EST2 is a catalytic subunit of telomerase. EST2p is capable of adding nucleotides to the 3'end of the single-stranded DNA using associated an RNA subunit as a template. Loss of *EST2* function leads to the loss of telomerase activity and telomere shortening (2).

Ustilago maydis is a fungus, which belongs to the order *Ustilaginales*, phylum *Basidiomycete* – this phylum includes common mushrooms. *Ustilago maydis* is a plant parasite preferentially infecting maize (corn) (Fig. 1). *U. maydis* exhibits three forms in its life cycle. It may exist either as a non-pathogenic unicellular haploid form, a dikaryotic filamentous parasitic form, and diploid teliospore, sometimes called spores (Fig. 2). Teliospores are the dormant form of the fungus, and they will germinate under appropriate conditions, producing haploid cells through meiosis. (1).

The goal of this project was to create a model system that imitates cancer cells with mutation in the *BRCA2* gene and inactive telomerase. We used a fungal plant parasite *Ustilago maydis* as our model organism as it carries both the homolog of *BRCA2* gene called *BRH2* and *EST2* gene, which codes for a catalytic subunit of telomerase (3).

Figure 1
Corn plants infected with *U. maydis*

Young corn plants infected with the fungus *Ustilago maydis*. The line indicates a tumor caused by *U. maydis* infection of the leaf.

Materials and Methods.

Sporulation

Sporangia were collected from a corn plant (Fig.2), dried, grounded up, and suspended in YEPD (Yeast extract/peptone/dextrose broth).

3 µl of suspension containing 104 spores were plated on YEPD media supplemented with 30 µl of ampicillin (60 µg/ml) and 25 µl of tetracycline (15 µg/ml). The spores were then incubated for 48 hours at 32^{0}C and, presence of microcolonies was verified under the microscope. Microcolonies were washed off the plate with 1 ml of YEPS (yeast extract/peptone/sucrose broth). Collected cell suspension was vortexed and the cell concentration was determined. 400 cells were plated on YEPD media, and the rest was frozen in 15% glycerol/YEPS.

Figure 2
Ustilago maydis sporangia on a corn plant.

Part of a corn stem with *Ustilago* sporangia grown on it was cut from the infected plant.

Cells plated on YPED media were incubated for 2 days at 32^{0}C and replica plated on various selection media.

Direct Selection

The plate to be replicated was marked to idicate a specific orientation; this same mark was made on the replicas. Afterwards, sterilized velveteen was carefully placed over an aluminum replica block, which was held in place by a rubber band. The original plate was opened, agar softly pressed against the surface of the velveteen, and the plate was removed. Fresh selection plates and the control plate were oriented the same way the original plate was, and the process was repeated for each plate. The plates were incubated for 2 days at 32^{0}C, and results were analyzed.

Preparation of Selelction Media

Selection media was prepared by supplementing YEPD with either 100 µg/ml of hygromycin (Hyg), 40 µg/ml of nourseothricin (Nat), or the combination of both.

UV – irradiation

Single colony grown on a solid media was inoculate in 3 ml of YEPD and incubated overnight on a rotor at 32^{0}C. After calculating cell concentration, cell cultures were diluted to the concentration of about 20 cells/µl. 10 µl of each cell suspension was spotted on two YEPD plates.One plate was exposed to UV-radiation for 2 minutes, while the second plate served as a control. Both plates were than wrapped in paper towels to prevent exposure to light and incubated at 32^{0}C for 48-hours.

DNA isolation

DNA was isolated from overnight culture by first disrupting cells in 10 mM Tris (tris(hydroxymethyl)amino methane), pH 7.5/0.1% SDS (sodium dodecyl sulfate) bufffer, and then removing proteins by phenol extraction. The aqueous phase was carefully collected, and genomic DNA was precipitated by isopropanol, dried and re-suspended in 10mM Tris, pH8.0/5mM EDTA (ethylenediaminetetraacetic acid) solution.

Polymerase Chain Reaction (PCR)

The reaction mix for PCR was prepared by mixing 264.5 µl of water, 37.5 µl of 10 x PCR buffer supplied by manufacturer, 30 µl of 25mM $MgCl_2$, 7.5 µl of 40 mM primer *Est2 DDI for 1*, 7.5µl of 40 mM primer *Est2 DDI rev1,* 7.5µl of 40 mM primer *Hyg DD rev1*, 9.4µl of 10 mM dNTP, and 3.75µl of Taq DNA polymerase.The reaction mix was then distributed evenly in 15 PCR tubes, 24.5 µl per each tube. 0.5µl of different genomic DNA samples was added per tube. The ingredients in the tubes were mixed by gently tapping the tube. The tubes were then placed in the PCR machine and the cycle was commenced.

The PCR cycle was as follows:
94^{0}C for 10 minutes to denature DNA,
Thermocycle:
94^{0}C for 1 minute; 55^{0}C for 2 minutes; 72^{0}C for 3 minutes repeated 25 times. This was follwed by incubation at 72^{0}C for 10 minutes, after which the temperature was decreased to 4^{0}C until the sample was recovered for analysis.

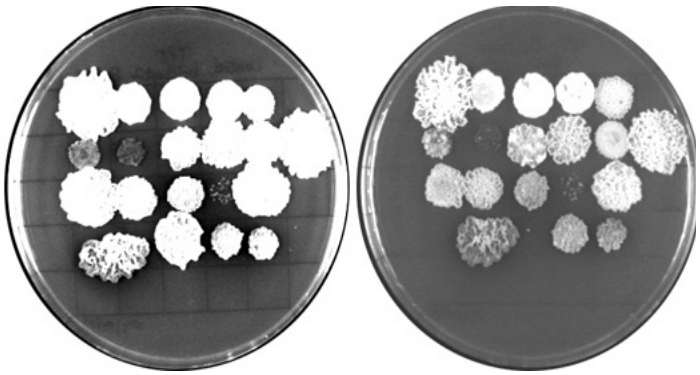
Cell counting
5 µl of the sample containing the cell/spore culture was placed on the grid of Neubauer hemocytometer. For each sample, five squares arranged diagonally were counted in anticipation of getting a fairly even accurate approximation. Cell count was performed in duplicates to ensure accuracy.

Results

est2 strain of *U. maydis* is resistant to Hyg (hygromycin), and *brh2* strain of this organism is resistant to Nat (nurseothricin) antibiotic. With this knowledge, it was expected that any cells that grew on Nat containg plate are *brh2 ,* and any cells that grow Hyg containg plate were *est2*. It is expected that any colony that grew on a media supplemented with both antibiotics is a double mutant *brh2/ est2.*

Extreme sensitivity to UV-radiation results from the loss of the *BRH2* gene function. To verify *brh2* genotype of strains grown on Nat and Hyg+Nat containing media, individual colonies from these plates were collected and analyzed for their sensitivity to UV-radiation (Fig. 3).

Figure 3
UV sensitivity of isolates strains.



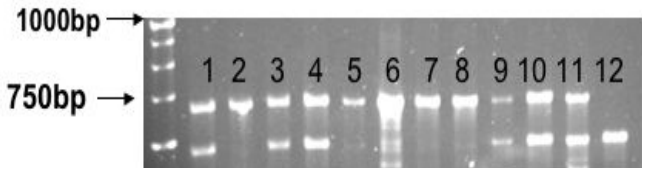
About 200 cells of each tested strain were spotted on two YEPD plates. One of the plates (A) served as a control, and the second one (B) was subjected to UV-irradiation for 2 minutes.Plates were wrapped in paper towels and incubated 2 days at 30^{0}C.

When comparing the UV-irradiated plate to the control plate, it can be seen that some isolates grew on the control plate (A-plate), but were not able to recover after UV treatment (B-plate).

Three strains carrying disruption of the *BRH2* gene were recovered from this experiment (Fig. 3).

Disruption of the est2 gene was verified by the PCR analysis. We used three different primers: *EST2 for DD1* and *EST2 rev DD1* were used to amplify *EST2* fragment of about 500 bp, and *EST2 for DD1* and *Hyg Rev DD1* were supposed to amplify fragment of the disrupted EST2 gene of about 750 bp (Fig 4).

Figure 4
Gel electrophoresis of PCR products



PCR reaction was performed as described in Materials and Methods. 5 µl of PCR reaction was loaded on 0.75% agarose gel and separated by electroporesis. Lanes 12 is a control (wild type *EST2* PCR products).

As seen on Figure 4, genomic DNA isolated from cells of samples 2, 5, 7, and 8 contained disruption of the *EST2* gene (*est2* strains).

Discussion.

Using direct selection followed by genotype verification based on sensitivity to UV radiation and presence/absence of the wild type gene in genomic DNA, we were able to isolate one *brh2/est2* strain, four *brh2/EST2* strains and three *BRH2*/*est2* strains of *Ustilago maydis*. When more of *brh2est2* strains are isolated, it will be possible to compare their viability and UV sensitivity to *brh2/EST2* and *BRH2*/*est2* strains, so to conclude about the effect of telomerase loss in *brh2* background with respect to these cells ability to survive.

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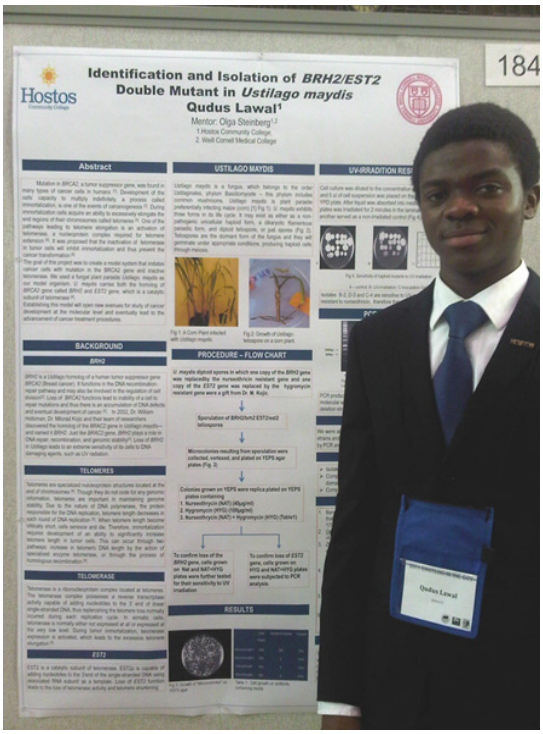
Aknowlegment.

Author expresses his graditude to the LSAMP program for providing financial support, to Dr. Neal Lue for the opoortunity to use his reserach facilities for implementation of the project, to Dr. Milorad Kojic for advice on the genetics of *Ustilago maydis,* and to Professor Olga Steinberg Neifach for overseeing the project.



Qudus Lawal presents the poster based on his research at the 2011 *Urban University Series /Einsteins in the City* Conference, City College, CUNY, April 14, 2011.

Acid-Fast Staining

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INTRODUCTION

Acid-fast stain, a type of differential stain, is primarily used to confirm the presence of pathogenic bacteria, *Mycobacterium tuberculosis*, and *Mycobacterium leprae,* the causative agents of tuberculosis and leprosy respectively. The common type of acid fast bacteria is Mycobacteria. Acid-fast bacteria contain within their cell walls a waxy material called mycolic acid, which gives them the property of resistance to destaining of basic dyes by acid-alcohol. During the Acid-fast staining procedure, Kinyoun method was used in which carbolfuchsin (primary stain) is applied to fixed smear in order to stain all the bacteria. Acid-alcohol (decolorizing agent) is used to remove the stain from the surface of the bacteria cell walls that do not bind to the stain. Finally, Brilliant green (counterstain) is used to counterstain the bacteria cells with a blue color contrasting to red acid-fast stain cells.

TERMINOLOGIES USED:

KINYOUN METHOD: also referred to as the cold method due to no application of heat.

STAINING: Is simply a procedure of coloring microorganisms with a dye that emphasizes certain structures.

ACID-FAST REACTION: a staining reaction in which organisms resist decolorization with acid alcohol and retain the primary dye.

NON-ACID FAST REACTION: a staining reaction in which organisms are susceptible to decolorization with acid alcohol and retain the counterstain.

MYCOLIC ACIDS: a waxy material comprising of long-chain fatty acids found in the cell wall of bacteria.

DIFFERENTIAL STAINS: are stains that react differently with different kinds of bacteria and can be used to distinguish them.

PRIMARY STAIN: a dye that imparts color to all cells. enzymes.

DECOLORIZING AGENT: a solution such as acid-alcohol that removes a stain from cells of some species and retains others.

COUNTERSTAIN: are stains that have a contrasting color to the primary stain.

PATHOGENS: refers to disease causing organismshydrolytic.

OBJECTIVES

To differentiate between the different groups of bacteria based on their cell wall composition.
To determine bacteria that are acid resistant and those that are non-acid resistant.

MATERIALS

Solid cultures of:
- *Staphylococcus aureus*
- *Mycobacterium smegmatis*

Acid fast staining set
- Carbolfuchsin
- Acid-alcohol
- Brilliant Green

PROCEDURE

The prepared heat-fixed smears were covered with carbolfuchsin for five (5) minutes.
Rinsed off with water for five (5) secs and drained the slide.
Decolorized with acid-alcohol for three (3) minutes.
Rinsed, drained, and counterstain with brilliant green for four (4) minutes.
Rinsed and blotted with bibulous paper.
Examined all preparations with the compound light

RESULTS AND OBSERVATIONS

Staphylococcus aureus stained red with carbolfuchsin, turned colorless with the application of acid-alcohol, then blue after being stained with brilliant green. *Mycobacterium smegmatis* stained red with carbolfuchsin, retained the red dye after application of both acid-alcohol and brilliant green.

CONCLUSIONS

The results of the experiment identified *Staphylococcus aureus* as non-acid fast bacteria due to their inability to retain the primary dye. This indicates the absence of mycolic acid in their cell walls. *Mycobacterium smegmatis* are acid fast bacteria indicating the presence of mycolic acid in their cell walls due to their primary dye retention ability.

Editor's note:

The laboratory report above is published the way it was originaly submitted by Ms. Odoom as a class assignment for the Microbiology course (BIO310). No changes, except where it was reqiered by transferring the document from Word into the publishing program, were made in the original manuscript.

Dear Reader,

Welcome to the third issue of the *Hostos Journal of Student Research* (HJSR)

The following are manuscript guidelines:

Scientific paper (definition).
Scientific paper has to be an original work by a student(s) composed under the mentorship of a faculty member of the Hostos Natural Sciences Department. A mentor's responsibility is to verify data and facts presented in the manuscript, and to avoid possible plagiarism by verifying citation references. Authorship of the paper should reflect the individual contributions of a student/students participating in the implementation of the research project. A mentor has to be included as one of the manuscript's authors.

Scientific essay/literature review (definition).
Scientific essay/literature review has to be an original work by a student submitted to a faculty member of the Hostos Natural Sciences Department. It is the faculty member's responsibility to verify facts presented in the essay, to make sure all conclusions of the essay are supported by scientific data, and to avoid possible plagiarism by verifying citation references. The name of the faculty member who helped the student with writing and editing the manuscript should appear next the student's name.
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All submissions are to be made via e-mail. Requests for publication should come from the student in the form of a cover letter e-mailed to any member of the editorial board with the scientific manuscript attached in MS Word format. **Figures and charts in JPEG format with legends in MS Word format should be sent as separate files**. Contact information for both the student and the mentor should be included in the manuscript. Scientific papers and essays previously published/copyrighted elsewhere will not be accepted for publication in the *Hostos Journal of Student Research.*

All manuscript should have an abstract. Abstract is a short summary (less than 200 words) of ideas and conclusions included in the scientific paper.

All references have to conform to the APA standard.

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Dear Editorial Board of the *Hostos Journal of Student Research*,

I would like to submit my manuscript (essay) titled…….., authors……..
for publication in the Natural Sciences Department on-line journal *Hostos Journal of Student Research.*
All questions considering the context of the manuscript (essay) should be addressed to me. My contact information……

Thank you for your consideration,
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Front page photographs:

Morphology of M-Ras KO astrocytes. (A) Immunofluores cence analysis of WT (_/_) and M-Ras KO (_/_) astrocytes. Astrocytes
are stained with a Cy3-conjugated anti-GFAP antibody (red), and
the actin cytoskeleton at the cell periphery was detected with an antiphospho-
ERM antibody (green). Two representative astrocytes are shown for each genotype. Magnification, X60.

Courtesy of Nelson Nunez-Rodriguez

Nuñez Rodriguez N., Lee I.N., Banno A., Qiao H.F., Qiao R.F., Yao Z., Hoang T., Kimmelman A.C., and A.M. Chan. 2006
Mol Cell Biol. 26:7145-54.