Evaluation of Methods for the Extraction and Purification of DNA from *Pseudomonas aeruginosa* and *Corynebacterium Xerosis*

Abstract:

The procedures to isolate of genetic material found in bacteria were a revolutionary breakthrough for researchers throughout the world. To examine the effectiveness of these procedures is required constant evaluation. Two bacteria, *Pseudomonas aeruginosa* and *Corynebacterium Xerosis*, were extracted with six different methods using boiling, freezing, or chemical methods before being run through a Nanodrop to determine the quality and quantity of the bacteria. Once done, the bacteria are run through an Agarose Gel Electrophoresis to confirm the bacterial DNA amount and concentration. The results were carefully calculated and documented. The results were repeated several times to determine quality of the experiments. In all cases, the already prepared QuickExtract Bacterial DNA Extraction kit produces the most desirable results for DNA extraction.
Background Information:

Seeing that bacteria make up a large proportion of the species of organisms on Earth, thousands of research studies in the last century were dedicated to observe and record these organisms. Only in the last few decades have scientist made the discovery that there is an estimate of 5 million trillion trillion bacteria in the world (Schloss, Handelsman 2004). A key aspect of why the knowledge of bacteria is important is that bacteria were among the first life on Earth. The oldest life form on Earth were prokaryotes and cyanobacteria, single celled bacteria, that were responsible for creating Earth’s first supply of oxygen 2.5 billion years ago (Hollar 2012). Since then, bacteria have played an important role in Earth’s ecosystem ranging from creating acids, food production, causing diseases, or spreading genetic information to one another.

Bacteria exist everywhere, even inside the human body. It is also stated that of the cells in the human body are roughly ninety percent are bacteria while ten percent are actually human cells (National Institutes of Health 2012). Despite the human cells being larger than the bacteria, bacteria greatly outnumbered them since they are tiny and can be compacted into smaller areas.

Like all other living cells, bacteria information is carried by DNA. DNA is essentially the building blocks of the bacteria and all other living organisms. Prokaryotic, bacteria, differ from Eukaryotic, all other life forms, is that all of bacteria genetic information is stored in one chromosome that are exactly copied by their mRNAs, a molecule in charge of creating protein from DNA (Rogers 2011). This genetic information is vital for information regarding the likelihood of the bacteria causing disease, transmitting to different hosts, or even adapting to antibacterial medicine. Bacteria can use binary fission to create offspring through sexual reproduction equipping new generations of bacteria with features that will help them survive in areas the previous generations could not (Rogers 2011).

Studying genomes remain crucial for the advancement of society. The foundation of genetic engineering is to modify microorganism is to produce products that would not be produce in nature, such as antibiotics, drugs, or nutrients (Maczulak 2011). Rearranging the nucleic acids in the DNA sequence inside bacteria is how scientists created artificial insulin for diabetic patients. The components extracted from bacterial genes can be used by biologists to identify the pathogens responsible for biological warfare (Hoyle 2004). The data found within the genes determines the ability of the pathogen to cause such destruction and holds the potential needed for researchers to find vaccines or antibiotics to combat these organisms. The first step to accomplish this feat is to first identify the components of the bacteria by extracting genetic information from the source.

In this experiment, DNA extractions from several bacterial species were used to determine the effectiveness of each procedure. DNA from different strains of bacteria was extracted to determine the purity and the amount of bacterial DNA yield for each extraction method. This research will help to determine the proper DNA extraction technique to use when gathering genetic information on microorganisms. From these DNA samples, scientist can use pure samples of DNA to test other factors for bacteria.

Research Question:

My research question is which bacterial DNA extraction process is the most effective in producing bacterial DNA base on the quality and quantities of the DNA produce.

Hypothesis:
I believe that Freezing and Thawing method will produce the most amount of bacterial DNA because undergoing constant stress such change in temperature cause many bacterial species to lyse and release DNA. It may be discover that extreme conditions will motivate the bacteria to find new ways to adapt and survive in their environment. There is a possibility that friction and movement may be just enough to get DNA. The possibilities are endless. The independent variables are the extraction techniques used and the dependent variables are the mass and purity of the DNA collected.

Variable Table:

<table>
<thead>
<tr>
<th>Name</th>
<th>I/D/C</th>
<th>Symbol</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction</td>
<td>I</td>
<td>V</td>
<td>mL</td>
<td>Each extraction process requires different amount of volume, and temperature in order to work.</td>
</tr>
<tr>
<td>Bacterial Species</td>
<td>C</td>
<td>T</td>
<td></td>
<td>Two bacterial species were constant throughout the procedure.</td>
</tr>
<tr>
<td>Mass</td>
<td>D</td>
<td>m</td>
<td>µg</td>
<td>The amount of bacterial DNA discovered is dependent on how the process was done.</td>
</tr>
</tbody>
</table>

Figure 1. Chart of all the variables.
Apparatus:

Figure 2. The Scale use to weigh the Agarose. The center button resets the weight to zero before weighing the object.

Figure 3. The Agarose Gel Electrophoresis is use to prepare the bacteria to be view on the computer. The buttons on the top of the machine controls volt, amps, and time. The buttons at bottom are the on switch.
Figure 4. The computer, Nanodrop, and Gel Doc EZ Imager (from left to right). The computer allow users to views the images produced by the other two machines. The Nanodrop has a moveable head that allows users to place sample of buffers. The Gel Doc Imager has an on switch by the side and a green button on front to measure the bacterial sample.

**Procedure:**

While creating the bacterial samples, the bacteria must incubated inside a broth solution overnight at 37℃ before starting each procedures.

1. **Boiling Protocol**
   a. Pipette 1 ml of bacterial sample into a 2 ml microcentrifuge tube.
   b. Boil the tube for 10 minutes at 100℃.
   c. Centrifuge the tube at 3,000 rpm for 4 minutes and keep the liquid.
   d. Add 1 ml of isopropyl alcohol and centrifuge the tube at 10,000 rpm for 4 minutes.
   e. Add 100 µl of ethanol alcohol and centrifuge again at 10,000 rpm for 4 minutes.
   f. Air dry the tube and add 100 µl of the TE Buffer.

2. **TE Boiling Protocol**
   a. Pipette 1 ml of bacterial sample into a 2 ml microcentrifuge tube.
   b. Centrifuge the tube at 3,000 rpm for eight minutes and keep the supernatant.
   c. Add 200 µl of TE Buffer into the microcentrifuge tube before boiling the tube at 100℃ for 10 minutes.
   d. Centrifuge the bacteria at 10,000 rpm for 4 minutes.
   e. Keep the liquid.

3. **TE Freeze and Thaw Protocol**
   a. Pipette 1 ml of bacterial sample into a 2 ml microcentrifuge tube.
   b. Centrifuged the tube at 3,000 rpm for 4 minutes.
   c. Discard the supernatant before adding 100 µl TE Buffer.
   d. Vortex the microcentrifuge tube.
e. Place the tube in a warm bath of 100°C for 1 minute before placing it in an ice water bath for 3 minutes, and then place in a warm bath of 100°C for an additional two minutes. Repeat this warm-cold-warm bath process for an additional two more times.

f. Centrifuged the tube a final time at 10,000 rpm for 4 minutes and keep the liquid in the tube.

4. Lysis Protocol
   a. Take a 1 ml sample of the bacteria and pipette the sample into a microcentrifuged tube before centrifuging it at 3,000 rpm for 10 minutes.
   b. Remove the liquid from it.
   c. Add 100 µl of lysis buffer into the tube and boil the tube into a warm bath of 100°C for 10 minutes.
   d. Centrifuged at 10,000 for 4 minutes and keep the liquid inside the tube.
   e. Add 100 µl of isopropyl alcohol and gently vortex the tube.
   f. Centrifuge the tube one more time at 10,000 rpm for 4 minutes.
   g. Remove the liquid and air-dry the tube for several seconds before adding 100 µl of TE Buffer.

5. Meat Tenderizer Protocol
   a. Take a 1 ml sample of the bacteria and pipette the sample into a microcentrifuge tube before centrifuging it at 3,000 rpm for 10 minutes.
   b. Remove the liquid from it.
   c. Add 10 µl of 20% solution of Meat Tenderizer Sample and incubate the sample at 37°C for 10 minutes. Centrifuge the sample at 10,000 rpm for 4 minutes.
   d. Keep the liquid.
   e. Add 100 µl of isopropyl alcohol and gently vortex the tube.
   f. Centrifuge the tube one more time at 10,000 rpm for 4 minutes.
   g. Remove the liquid and air-dry the tube for several seconds before adding 100 µl of TE Buffer.

6. KIT Protocol
   a. Pipette 1 ml of bacterial sample into a 2 ml microcentrifuge tube.
   b. Centrifuge the tube for 3 minutes at 5,000 rpm.
   c. Remove supernatant.
   d. Add 0.5 ml of sterile water to the sample before microcentrifuging it again for 3 minutes at 5,000 rpm.
   e. Remove the liquid.
   f. Add 100 µl of QuickExtract Bacterial DNA Extraction solution.
   g. Add 1 µl of Ready-Lyse Lysosyme Solution to the tube before the sample is vortex.
   h. Incubate the sample at 37°C for 15 minutes.

Using the NanoDrop:
   a. Click on the NanoDrop™ 2000 software icon.
   b. Select the Nucleic Acid option.
   c. Write the name for the new data chart.
   d. Wipe the NanoDrop™ pedestal with a laboratory wipe.
e. Pipette 2 µl of TE Buffer into the NanoDrop™ pedestal.
f. Click the Blank button.
g. Wipe away the blank.
h. Pipette 2 µl of one DNA sample into the NanoDrop™ pedestal.
i. Click the Measure Button.
j. Wipe the NanoDrop™ pedestal with a laboratory wipe again.
k. Click on the Blank Button and repeat step e to step j until all bacterial samples have been documented.
l. From there, a person can save a pdf file of their documents.

Making the Gel:
   a. Set up the gel holder at the ends of the DNA tray.
   b. Add one gel comb.
   c. Take a 250 ml flask and add 10 ml of TAE Buffer
d. Measure 0.5 grams of agarose on a scale and pour it into the flask.
e. Cover flask with saran wraps and microwave it for 2 minutes.
f. Swirl the liquid around every 30 seconds until agarose dissolved.
g. Let the flask cool for 5 minutes before pouring the liquid into the gel tray.
h. Let the gel tray cool for 15 minutes so the gel can harden.

Loading the Gel:
   a. Once the gel hardens, remove the gel tray.
   b. Make sure the DNA equipment is face in a direction where the red side will face the machine.
   c. Pour enough TAE Buffer to cover the entire gel.
   d. Take a clean piece of parafilm and add 1.5 µl of SYBR® Green, 1.5 µl of loading dye, and 5 µl of bacterial sample. Mix the products by pipetting the three ingredients and placing it back on the parafilm. Repeat this step for all bacterial samples.
   e. Place each mixture on corresponding lanes within the gel tray.
   f. Attach the black and red wires to the appropriate outlets on the machine.
   g. Set the machine to 95 V for 35 minutes.

Viewing the Gel:
   a. Open the Gel Doc™ EZ imaging system.
   b. Remove the Ultraviolet Sample Tray.
   c. Clean the tray with water and wipe it dry with a laboratory wipe.
   d. Place the gel sample onto the tray and place the tray back into the machine.
   e. On the system.
   f. Select the SYBR® Green Button.
   g. Print and view file.
Data and Analysis:

<table>
<thead>
<tr>
<th>#</th>
<th>Sample ID</th>
<th>Nucleic Acid Quantity</th>
<th>Unit</th>
<th>A260 (Abs)</th>
<th>A280 (Abs)</th>
<th>260/280 Quality</th>
<th>260/230</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pseudomonas</td>
<td>70</td>
<td>ng/µl</td>
<td>1.401</td>
<td>0.953</td>
<td>1.47</td>
<td>0.42</td>
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<tr>
<td></td>
<td>Boiling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas</td>
<td>868.8</td>
<td>ng/µl</td>
<td>17.377</td>
<td>12.281</td>
<td>1.41</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>TE Boiling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas</td>
<td>430</td>
<td>ng/µl</td>
<td>8.6</td>
<td>5.962</td>
<td>1.44</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>TE F/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas</td>
<td>81.5</td>
<td>ng/µl</td>
<td>1.629</td>
<td>1.727</td>
<td>0.94</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Lysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas</td>
<td>897</td>
<td>ng/µl</td>
<td>17.939</td>
<td>12.345</td>
<td>1.45</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>M/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td>Pseudomonas</td>
<td>315.4</td>
<td>ng/µl</td>
<td>6.307</td>
<td>4.055</td>
<td>1.56</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>KIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. The table shows all the data taken from the NanoDrop on Pseudomonas Aeruginosa. Each column bacterial sample is given a DNA extraction technique. The Nucleic Acid Quantity column showed the amount of Pseudomonas aeruginosa generated from each technique. The unit of measurement is ng/µl. The A260 column determines the absorbance of RNA concentration while A280 determines the protein concentration. The ratio of the two numbers is the purity of the DNA sample. The ideal number for this ratio is 1.8 and numbers below 1.8 expressed DNA contaminations. 260/230 is a second ratio used to measure DNA purity. The ideal number 2.0 and numbers below that also expressed DNA contaminations.
Figure 7. The ratio 260/280 of the Pseudomonas aeruginosa collected to dictate the purity of the DNA samples. The ratio of A260/A280 measures the absorbance of RNA while A260 determines the wavelength. Two previous attempts at a Lysis was made and improperly recorded.

Figure 8. The Pseudomonas aeruginosa image taken from the Gel Electrophoresis Image. This showed the amount of DNA found in each lane. The first lane is the Boiling technique. The second is the TE Boiling technique. The third lane is TE Freeze and Thaw technique. The fourth lane is Lysis technique. The fifth lane is the Meat Tenderizer technique. The sixth lane is the KIT technique.

Since nucleic acid represents the amount of DNA produced, the TE Boiling method produced the most amounts of DNA from the Pseudomonas aeruginosa. The Boiling method gave the least amount of DNA. When it comes to quality of DNA from Pseudomonas aeruginosa however, the Boiling technique gave the purest DNA sample, while the Lysis gave the least.
<table>
<thead>
<tr>
<th>#</th>
<th>Sample ID</th>
<th>Nucleic Acid Quantity</th>
<th>Unit</th>
<th>A260 (Abs)</th>
<th>A280 (Abs)</th>
<th>260/280 Quality</th>
<th>260/230 Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arane Corynebacterium Boiling</td>
<td>127.8 ng/µl</td>
<td></td>
<td>2.557</td>
<td>1.652</td>
<td>1.55</td>
<td>0.44</td>
</tr>
<tr>
<td>2</td>
<td>Arane Corynebacterium TE Boiling</td>
<td>805.4 ng/µl</td>
<td></td>
<td>16.107</td>
<td>11.588</td>
<td>1.39</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>Arane Corynebacterium TE F/T</td>
<td>343.5 ng/µl</td>
<td></td>
<td>6.87</td>
<td>4.871</td>
<td>1.41</td>
<td>0.44</td>
</tr>
<tr>
<td>4</td>
<td>Arane Corynebacterium Lysis</td>
<td>328.3 ng/µl</td>
<td></td>
<td>6.566</td>
<td>4.66</td>
<td>1.41</td>
<td>0.44</td>
</tr>
<tr>
<td>5</td>
<td>Arane Corynebacterium M/T</td>
<td>369.7 ng/µl</td>
<td></td>
<td>7.394</td>
<td>5.378</td>
<td>1.37</td>
<td>0.44</td>
</tr>
<tr>
<td>6</td>
<td>Arane Corynebacterium KIT</td>
<td>358.9 ng/µl</td>
<td></td>
<td>7.178</td>
<td>5.236</td>
<td>1.37</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Figure 9. The table shows all the data taken from the NanoDrop on Corynebacterium Xerosis. Each column bacterial sample is given a DNA extraction technique. The Nucleic Acid Quantity column showed the amount of Corynebacterium xerosis generated from each technique. The unit of measurement is ng/µl. The A260 column determines the absorbance of RNA concentration while A280 determines the protein concentration. The ratio of the two numbers is the purity of the DNA sample. The ideal number for this ratio is 1.8 and numbers below 1.8 expressed DNA contaminations. 260/230 is a second ratio used to measure DNA purity. The ideal number 2.0 and numbers below that also expressed DNA contaminations.
Figure 10. The ratios of the Corynebacterium Xerosis collected. The ratio 260/280 of the Corynebacterium Xerosis collected to dictate the purity of the DNA samples. The ratio of A260/A280 measures the absorbance of RNA while A260 determine the wavelength. Two previous attempts at a Lysis was made and improperly recorded. An incorrect attempt of a Freeze and Thaw was recorded into the data.
Figure 11. The Corynebacterium xerosis image taken from the Gel Electrophoresis Image. This showed the amount of DNA found in each lane. The first lane is the Boiling technique. The second is the TE Boiling technique. The third lane is TE Freeze and Thaw technique. The fourth lane is Lysis technique. The fifth lane is the Meat Tenderizer technique. The sixth lane is the KIT technique.

The TE Boiling method produces the most amounts of DNA from Corynebacterium xerosis. The Boiling method gave the least amount of DNA. When it comes to quality among the Corynebacterium xerosis however, the Boiling technique gave the purest DNA sample, while the Meat Tenderizer method gave the least.

Conclusion:

My previous hypothesis was incorrect. The Freeze and Thaw technique did not produce the best or even the most DNA. In both instances, the Boiling Protocol produce the best quality of bacterial DNA when compare to the other techniques. The TE Boiling, however, produced the most bacterial DNA. Even in current times, there are varieties of methods to choose from when it pertains to DNA extractions. Despite DNA extraction playing an important role the studies of microorganisms, there are no established standard for DNA extraction (Psifidi et al., 2015). There are no established reports dictating any methods are more superior to the other methods. Scientists have the options to choose commercial kits to carry out their experiments or to use simpler methods such as heat shock. Long before commercial DNA extraction kits became available for researchers, heat extractions were the ideal methods use to collect DNA. Heat shock was preferred because it is a milder procedure than electroshock (Li et al., 2007). The problem with electroshock was that it often destroyed the entire cell while breaking down the cell
membrane to release DNA. The genetic information cannot withstand the high voltage required to penetrate the cell membrane. Studies conducted by Researchers Jose and Brahmadathan showed that heating at 94 °C for two minutes was enough to destroy cell walls (Dashti et al., 2009). The same research also showed that low temperature destroys cell walls because the crystallization of the water causes the cytoplasmic structures to break down. Their experiment suggests that the Heat and Thaw should have produced the most DNA. In this experiment, the amount of time the bacteria was in boiling water or freezing water could have been decreased. There is a possibility that the repetition of placing the sample in and out two baths destroyed some other genetic materials. This research was essential in the field of microbiology. It confirms that when bacteria come face to face with harsh conditions such as heat, the bacteria will leave behind DNA fragments. In the case of pathology, bactericide can kill off a microbe but if the DNA fragments are left behind then another strain of bacteria can easily incorporate the foreign DNA. This act, called transformation, is a cause of new and more dangerous strains of bacteria. Bacteria must be sterilized to reduce chances of this happening. In the field of genetic engineering, the bacterial DNA remains are quite promising for further research. With a proper technique to isolate DNA fragments, further studies can be made to identify the key proteins involved with enabling a bacteria’s virulence and other traits among the bacteria.
References


