2018

AP CollegeBoard

AP Research Academic Paper

Sample Student Responses and Scoring Commentary

Inside:

Sample D

- ☑ **Scoring Guideline**
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2018 AP Research Academic Paper Rubric **v1.0**

The response…

Score of 1 **Report on Existing Knowledge**	**Score of 2** **Report on Existing Knowledge with Simplistic Use of a Research Method**	**Score of 3** **Ineffectual Argument for a New Understanding**	**Score of 4** **Well-Supported, Articulate Argument Conveying a New Understanding**	**Score of 5** **Rich Analysis of a New Understanding Addressing a Gap in the Research Base**
• Presents an overly broad topic of inquiry.	• Presents a topic of inquiry with narrowing scope or focus, that is NOT carried through either in the method or in the overall line of reasoning.	• Carries the focus or scope of a topic of inquiry through the method AND overall line of reasoning, even though the focus or scope might still be narrowing.	• Focuses a topic of inquiry with clear and narrow parameters, which are addressed through the method and the conclusion.	• Focuses a topic of inquiry with clear and narrow parameters, which are addressed through the method and the conclusion.
• Situates a topic of inquiry within a single perspective derived from scholarly works **OR** through a variety of perspectives derived from mostly non-scholarly works.	• Situates a topic of inquiry within a single perspective derived from scholarly works **OR** through a variety of perspectives derived from mostly non-scholarly works.	• Situates a topic of inquiry within relevant scholarly works of varying perspectives, although connections to some works may be unclear.	• Explicitly connects a topic of inquiry to relevant scholarly works of varying perspectives **AND** logically explains how the topic of inquiry addresses a gap.	• Explicitly connects a topic of inquiry to relevant scholarly works of varying perspectives **AND** logically explains how the topic of inquiry addresses a gap.
• Describes a search and report process.	• Describes a nonreplicable research method **OR** provides an oversimplified description of a method, with questionable alignment to the purpose of the inquiry.	• Describes a reasonably replicable research method, with questionable alignment to the purpose of the inquiry.	• Logically defends the alignment of a detailed, replicable research method to the purpose of the inquiry.	• Logically defends the alignment of a detailed, replicable research method to the purpose of the inquiry.
• Summarizes or reports existing knowledge in the field of understanding pertaining to the topic of inquiry.	• Summarizes or reports existing knowledge in the field of understanding pertaining to the topic of inquiry.	• Conveys a new understanding or conclusion, with an underdeveloped line of reasoning **OR** insufficient evidence.	• Supports a new understanding or conclusion through a logically organized line of reasoning **AND** sufficient evidence. The limitations and/or implications, if present, of the new understanding or conclusion are oversimplified.	• Justifies a new understanding or conclusion through a logical progression of inquiry choices, sufficient evidence, explanation of the limitations of the conclusion, and an explanation of the implications to the community of practice.
• Generally communicates the student's ideas, although errors in grammar, discipline-specific style, and organization distract or confuse the reader.	• Generally communicates the student's ideas, although errors in grammar, discipline-specific style, and organization distract or confuse the reader.	• Competently communicates the student's ideas, although there may be some errors in grammar, discipline-specific style, and organization.	• Competently communicates the student's ideas, although there may be some errors in grammar, discipline-specific style, and organization.	• Enhances the communication of the student's ideas through organization, use of design elements, conventions of grammar, style, mechanics, and word precision, with few to no errors.
• Cites **AND/OR** attributes sources (in bibliography/works cited and/or in-text), with multiple errors and/or an inconsistent use of a discipline-specific style.	• Cites **AND/OR** attributes sources (in bibliography/works cited and/or in-text), with multiple errors and/or an inconsistent use of a discipline-specific style.	• Cites **AND** attributes sources, using a discipline-specific style (in both bibliography/works cited AND in-text), with few errors or inconsistencies.	• Cites **AND** attributes sources, with a consistent use of an appropriate discipline-specific style (in both bibliography/works cited AND in-text), with few to no errors.	• Cites **AND** attributes sources, with a consistent use of an appropriate discipline-specific style (in both bibliography/works cited AND in-text), with few to no errors.

AP® RESEARCH
2018 SCORING COMMENTARY

Academic Paper

Overview

This performance task was intended to assess students' ability to conduct scholarly and responsible research and articulate an evidence-based argument that clearly communicates the conclusion, solution, or answer to their stated research question. More specifically, this performance task was intended to assess students' ability to:

- Generate a focused research question that is situated within or connected to a larger scholarly context or community;
- Explore relationships between and among multiple works representing multiple perspectives within the scholarly literature related to the topic of inquiry;
- Articulate what approach, method, or process they have chosen to use to address their research question, why they have chosen that approach to answering their question, and how they employed it;
- Develop and present their own argument, conclusion, or new understanding while acknowledging its limitations and discussing implications;
- Support their conclusion through the compilation, use, and synthesis of relevant and significant evidence generated by their research;
- Use organizational and design elements to effectively convey the paper's message;
- Consistently and accurately cite, attribute, and integrate the knowledge and work of others, while distinguishing between the student's voice and that of others;
- Generate a paper in which word choice and syntax enhance communication by adhering to established conventions of grammar, usage, and mechanics.

The Thin-Layer Chromatography Analysis of Phospholipids in *Vibrio fischeri* with a focus on Cardiolipin

Word Count: 4,242

TLC ANALYSIS OF CARDIOLIPIN IN *V. FISCHERI*

Abstract

Cardiolipin is found in bacterial and mitochondrial membranes, making it a compound that is found in virtually all organisms. While the physical properties of cardiolipin are known, most of the functions and significance of this phospholipid are simply hypothesized. In the case of *Helicobacter pylori* and *Vibrio fischeri*, cardiolipin is hypothesized to be essential for the production of flagellar sheaths and bacterial mobility. However, the absence of cardiolipin-producing genes in *V. fischeri* did not affect its motor function. As a result, I will be studying the presence of cardiolipin in multiple *V. fischeri* mutants with genes coding for cardiolipin removed in order to detect other possible genes responsible for cardiolipin production or to conclude that perhaps cardiolipin is nonessential for flagella synthesis. The method of analysis used for this research includes lipid extraction and thin-layer chromatography in order to compare the presence and relative concentrations of phospholipids present in *V. fischeri*. The results of the thin-layer chromatography interestingly revealed a high concentration of phosphatidylglycerol (PG); when comparing the structures of PG with cardiolipin, it is revealed that the hydrophilic heads of both phospholipids are similar. This suggests that *V. fischeri* is able to retain its flagellar sheaths and motor function due to PG undertaking the same functions of cardiolipin. This study increases the current body of knowledge of cardiolipin, allowing for a basis of further research.

Introduction

Flagellum is one of the most common mechanisms used by bacteria; the flagellum assists bacterium in swimming through liquid environments and invading then adhering to host cells. In some cases, the flagellum of the bacteria is responsible for the pathogenic and virulent characteristics. Bacterial flagella are made up a specific array of proteins, and some are selectively enclosed by a sheath that is often a continuation of the outer membrane. The purpose of the flagellar sheath is unclear, but it is suspected that the flagellar sheath assists in host-microbe interactions and can prevent the host's immune system from detecting the bacteria (Brennan et al., 2014). Two microbes that exhibit the flagellar sheath are *Helicobacter pylori* and *Vibrio fischeri*; both often studied for their use of the flagellum in order to enter and affect hosts.

In the lab where I am researching, the main focus of the lab is the flagellum localization of *Helicobacter pylori*; the lab focuses on researching the polarization of the flagella in *Helicobacter pylori*. The flagella in *Helicobacter pylori* are situated at the poles of the rod-shaped bacteria, and the lab has focused on the possibility of the phospholipid cardiolipin as the compound responsible for the location of the flagella and its accompanied sheath. *Helicobacter pylori* is a gram-negative bacterium that colonizes the human's gastric system and infects 50% of the population (Skene, Young, Every & Sutton, 2007). Skene, Young, Every, and Sutton (2007) had discussed that the motility of *H. pylori* is crucial for its successful colonization and virulent characteristics, seen by non-motile *H. pylori* mutants having unsuccessful infections in host cells. As a result of the consequences of *H. pylori*'s motility, it is essential to research what assists in producing the flagella and flagellar sheath that these virulent bacteria possess. The lab with which I am associated with is assessing the role that cardiolipin plays in flagellum localization.

However, due to the pathogenic nature of *Helicobacter pylori*, I investigated cardiolipin in the bacteria *Vibrio fischeri*; *V. fischeri* is a reliable alternative to study cardiolipin since it also possesses localized, polarized flagella with the unique flagellar sheath. In previous experiments, when cardiolipin-coding possible genes were removed from *V. fischeri,* the bacteria still retained its flagella and motility which was in contradiction with the idea that cardiolipin is essential for flagella. As a result, I will be studying the presence of cardiolipin in multiple *V. fischeri* mutants with genes coding for cardiolipin removed in order to detect other possible genes responsible for cardiolipin production or to conclude that perhaps cardiolipin is nonessential for flagella synthesis.

Literature Review

Vibrio fischeri is a gram-negative bacterium that is found in both seawater and a symbiotic relationship with certain squids; with its bioluminescence, *V. fischeri* colonizes the light organs of the host squid (Brennan et al., 2014). Brennan et al. (2014) explore the role that the sheathed flagellum plays in the symbiotic relationship between *V. fischeri*; their research showed that the bacteria that did not possess flagella or flagellar sheaths were not able to interact with the host squid. In this case, only the bacteria with "working flagella were able to trigger the normal development of the squid's light organ" (Brennan et al., 2014). Researchers Millikan and Ruby (2004) also studied the relationship between the sheathed flagellum that *V. fischeri* possesses and the colonization of the Hawaiian bobtail squids' light organs. In their research, it was discovered that out of the six subunits that compromise of the flagellum, the gene expression of the "subunit, *flaA*, is directly dependent on the motility master regulator" (Millikan & Ruby, 2004). While this demonstrates that there is a scientific body of knowledge around this particular symbiosis and flagellum construction, *V. fischeri* also possesses a component of some flagella that are not very common: a flagellar sheath. *V. fischeri* has a flagellar sheath that is an extension of the outer membrane which encases the flagellum necessary for motility and colonization of a host squid's light organ (E., 2016). The reasons and the components behind a flagellar sheath are widely hypothesized.

While the flagellar sheath is not very common among bacteria, the bacteria *Helicobacter pylori* also possesses this unique characteristic. The flagella that *H. pylori* has are unipolar, usually number three to five, and are covered by a sheath layer that appears to be "relatively fluid and membrane-liked judged by its electron microscopic appearance in negatively stained preparations" (Penn & Luke, 1994). The flagella and their corresponding sheaths are

hypothesized to be important for the motility of *H. pylori*; *H. pylori*, which is an extracellular, highly motile bacterium, "infects 50% of the human population worldwide and long-term infection is associated with the development of gastric and duodenal ulcers" (Skene, Young, Every, & Sutton, 2007). Skene et al. (2007) discussed that a vaccine could be possibly developed to focus the immune system on antigenic structures present in the flagellar sheath, yet "there has been very little investigation of membrane-bound flagellar sheath proteins." Another study focused on possible auto transporter proteins that enhance the capacity of *H. pylori* to infect the stomach. Surprisingly, one of these proteins (FaaA) is localized to the sheath present around *H. pylori* flagella; the absence of the protein FaaA "decreased *H. pylori* motility as well as a reduction in flagellar stability and a change in flagellar localization" (Radin et al., 2013). A compound that has been hypothesized and is currently being studied in association with flagellar sheaths is the phospholipid cardiolipin.

Cardiolipin is a phospholipid that is found in both bacterial and mitochondrial membranes, essentially making it a compound that is "found in virtually all organisms of the three domains of life: eubacteria, archaebacteria, and eukaryote" (Schlame, 2007). Cardiolipin belongs to a class of phospholipids, polyglycerophospholipids, in which the head group and backbone are formed from repeating subunits of phosphoryl and glycerol components (Schlame, 2007). Cardiolipin is "a large anionic glycerol phospholipid composed of four large acyl chains connected by a small glycerol head group"; this structure gives cardiolipin a conical shape which allows it to accumulate at membrane regions presenting negative curvature (Rossi, Yum, Agaisse, & Payne, 2017). While cardiolipin is essential for the maintenance of the highly curved regions of the bacterial and mitochondrial membranes, cardiolipin also provides spatial organization for the proteins needed in respiration and oxidative phosphorylation (Boyd, Alder,

& May, 2017). A study done by Sautrey et al. (2016) discusses how "amphiphilic aminoglycoside antibiotics acting on lipid domains, including cardiolipin clusters, could be promising antibacterial compounds" due to cardiolipin often being present in various Gram-negative bacteria that often cause illness in their hosts. Cardiolipin, due to its large presence in the negatively curved areas of cellular membranes, has also been hypothesized to be responsible for the spatial organization and construction of flagella or flagellar sheaths that are present in the same region as cardiolipin clusters. This hypothesis has been supported by "recent experiments that suggest CL domains may be responsible for polar protein localization in *E. coli* cells" (Renner & Weibel, 2011).

Methods

The purpose of the experiment is to complete a thin layer chromatography analysis of the various phospholipids—with a focus on cardiolipin—in the bacteria *Vibrio fischeri.* Thin layer chromatography will be used to analyze both the presence and relative concentrations of the phospholipids across the four strains of *V. fischeri* that are being studied.

Bacteria Strains

Four strains of *V.* fischeri are being used in this experiment; a wild-type strain, two strains with different genes removed, and a double mutant strain with the two genes removed that were removed in the single mutant strain. The wild-type strain, with no changes to its genome, will be labeled as WT throughout the experiment. The first single mutant is the strain VFS011E1 which has a knockout of the gene *ymdC*, which has the locus number VF0865. The protein encoded by *ymdC* has the highest homology with *Helicobacter pylori clsH* (cardiolipin synthase) of any *V. fischeri* protein. Basically, the gene removed in this strain produces a protein that is most similar to the protein in *H. pylori* which produces cardiolipin. This strain will be labeled as E1 in the following procedures. The second single mutant strain VFS022A8 has a knockout of *cls*, which has a locus number VF1606; *cls* is the gene that has been previously identified by scientists as a cardiolipin synthase. This single mutant strain will be labeled as 22A or A8. The genes that were removed in E1 and 22A have transposon insertions in the genes previously indicated. In a transposon insertion, a transposable element was inserted into the *V. fischeri* genome; this DNA sequence can change its position within the genome which often creates mutations and alters the bacterial cell's genetic identity. In the case of this experiment, the transposon did not allow for the expression of the genes *ymdC* (E1) and *cls* (22A). The double mutant strain JLS78A has an unmarked deletion of *ymdC* and transposon insertion in *cls*. As a

result, the bacteria is missing both of these genes and is not expressing them. This final strain will be labeled as 78A in the procedures and results.

Harvesting the Bacteria

Since *V. fischeri* is found in the light organ of a squid, an aquatic creature, the bacteria was grown in a Laurie Broth Salt media. No agar was used in the media to turn it solid since the media needed to remain as a liquid for the aquatic bacteria. The media was prepared in a large Erlenmeyer flask with one liter of water, twenty grams of sodium chloride (more than normal since the environment for the bacteria is saltwater), five grams of yeast extract, and 10 grams of tryptophan. The prepared media was autoclaved in order to destroy any endospores that may be present.

In order to inoculate the bacteria, a sterile stick was used to place on colony of the particular bacteria strain in a test tube containing approximately five mL of the media. After incubating this inoculation overnight at room temperature, place three mL of the inoculated mixture into the one-liter batch of media previously prepared. The flask containing the bacteria and solution was placed into a water bath shaker for 24 hours.

After sufficient growth, the bacteria and media solution will be centrifuged in order to harvest the bacteria for the next steps. After centrifugation, the solution will separate into a supernatant and pellet. The supernatant can be drained off since the pellet contains the bacteria needed for further lipid extraction. In order to preserve the quality of the bacteria, the pellet is frozen until the next step. The previously mentioned procedure is repeated for each of the four strains; all four strains should be harvested and prepared at the same instance in order to complete the thin layer chromatography in a comparative manner.

Lipid Extraction

The following method for total lipid extraction and purification is borrowed from proportions and solutions determined by Bligh and Dyer (1959). First, in order to prepare the bacterial sample from which the lipid extraction will occur from, the bacterial pellet previously harvested was diluted with distilled water. Next, 0.25 mL of the diluted bacterial culture and 0.75 mL of water were combined in a sterile tube and vortexed well for one minute. Then, 3.75 mL of 1:2 (v/v) $ChCl_3$:MeOH was added to the tube and vortexed for one minute. Next, 1.25 mL of $CHCl_3$ (chloroform) was added to the tube and once again vortexed for one minute. Finally, 1.25 mL of distilled H_20 was added to the tube and vortexed for one minute. Each time liquid was added to the tube and was vortexed, the speed of the vortex machine was incrementally turned down in order to prevent spills.

After the last vortex, the final solution in the test tube was centrifuged in order to separate the solution into an aqueous upper phase and an organic bottom phase. The lipids needed for the thin layer chromatography were present in the bottom phase. In order to recover the bottom phase, a Pasteur pipette was inserted through the upper phase with slight positive pressure in order to ensure none of the upper phase was accidentally retrieved. Once the pipette was at the bottom, the organic material was carefully extracted. Only 90% of the bottom phase was attempted to be recovered to once again ensure no upper phase was also extracted. The extracted organic material was further processed by transferring it into sterile test tubes in order to let the solvent evaporate. The previously mentioned procedure is repeated for each of the four strains; all four strains underwent lipid extraction together in order to complete the thin layer chromatography at the same time.

Thin-Layer Chromatography

Thin layer chromatography utilizes a thin layer of adsorbent material, in this this case silica gel, in order to separate compounds depending on their affinity for the solvent used. To prepare the extracted lipids for thin layer chromatography, the lipids were re-suspended in a total of 100 microliters of a solution comprised of 1:2 (v/v) $ChCl_3$:MeOH; the solution was added in portions of fifty microliters in order to ensure that all the extracted lipids were dissolved.

The thin layer chromatography plate is prepared by spotting the re-suspended lipid solutions of each of the four stains on the same plate in order to streamline the comparative analysis. On a sterile silica gel plate, draw a straight line approximately two inches above the bottom edge in order to ensure that the lipid solutions do not seep into the solvent and skew the results. Two microliters of one lipid solution was taken and spotted onto the line drawn. After allowing the spot to dry, another two microliters of the same lipid solution were spotted onto the same spot and allowed to air dry. This process was done two more times until a total of eight microliters of the same lipid solution was spotted. This spotting procedure was repeated for the other three lipid solution for the different strains, placing each strain's spot spaced out from each other. By having sufficient space between the spots of the lipid solutions, interference from other strains was controlled and results were not skewed. The prepared silica gel plate was placed into a closed glass chamber with a solution of chloroform: methanol: acetic acid in a ratio of 65:25:10. For clearer results, a filter paper soaked in the solution of chloroform: methanol: acetic acid was also placed alongside the silica gel plate; the soaked filter paper provided more opportunities for the solvent to run up the plate and provide optimal thin layer chromatography results.

After letting the solvent front run to about three-quarters to the top, the plate was removed and immediately sprayed with our lipid-staining reagent. The thin layer chromatography plate needed to be immediately sprayed after being taken out of the thin layer chromatography chamber in order to get clear and optimal results. The spray reagent that was used was a solution of ammonium pentachlorooxomolybdate and sulfuric acid (Kundu, Chakravarty, Bhaduri, & Saha, 1977). This reagent interacts with the lipids that had traveled up and stains them in a bright blue color. The stain allows for clear analysis of both presence and relative concentrations of the phospholipids.

Results

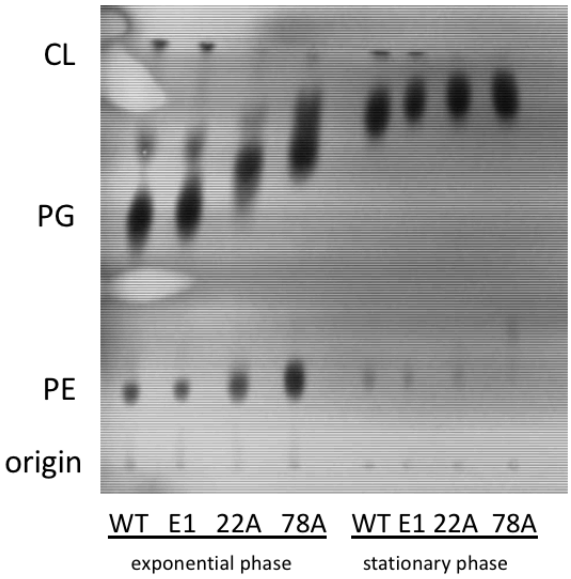

Figure 1: Thin layer Chromatography plate for exponential vs. stationary phases

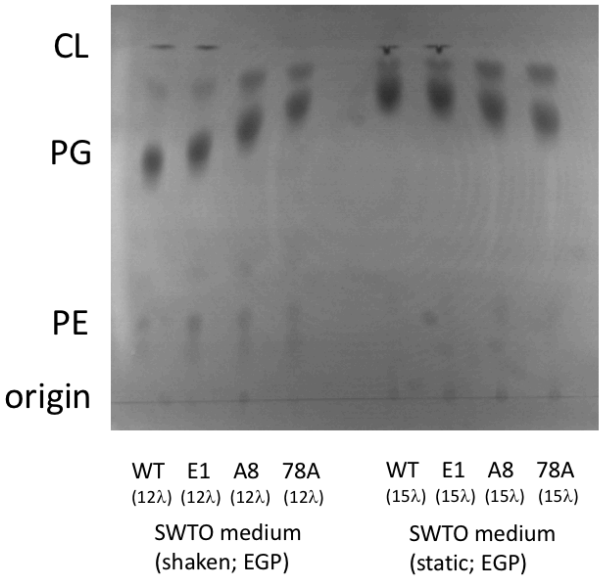

Figure 2: Thin layer Chromatography plate for shaken vs. static growth

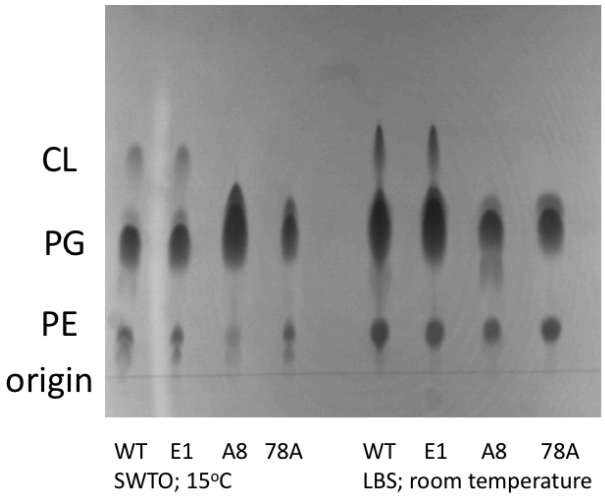

Figure 3: Thin layer Chromatography plate for 15 ºC vs. room temperature growth

Analysis

In all three figures above, it can be seen that the lipids present from the thin layer chromatography have separated into three different phospholipids: cardiolipin (CL), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE). The three lipids have been identified in their respective regions by current research being done by the University of Georgia. In this experiment, three different pairs of conditions were compared in order to create the ideal environment for the bacteria to grow and have the data show the best representation of the phospholipids present in *V. fischeri*. Three contrasting conditions were tested: growth phase (Figure 1), movement during growth (Figure 2), and temperature during growth (Figure 3). The conditions for the growth phase were determined by the length of time that the bacteria were allowed to grow. For the movement during growth, the shaken condition means that the bacteria in the medium was grown in a water bath shaker while the static condition meant that the batch of media and bacteria was grown on the lab counter. Finally, the temperature condition was implemented by changing the temperature in the environment where bacteria was grown. The default growth conditions for the bacteria were exponential phase, shaken media, and room temperature; as a result, the other two conditions remained the same while one condition was being varied.

Figure 1 compares the thin layer chromatography results between *V. fischeri* grown in the exponential phase and stationary phase. In bacterial growth, the exponential phase is a period of development characterized by cell doubling and the population of bacteria is significantly growing. The stationary phase is when the growth rate and death rate are equal and so the population of total bacteria remains steady; this is often due to a limiting-factor such as depletion of nutrients. For the exponential phase, it can be seen that all four *V. fischeri* strains show

presence of both PG and PE. However, only the strains WT and E1 demonstrated a presence of CL. From comparing the sizes of the spots in the respective regions of Cl, PG, and PE, it can be seen that PG is present in higher quantities than CL. The same trend in location of the phospholipid spots can be observed in the results of the stationary phase. The curvature that can be seen in the results of Figure 1 was a common result that can often occur in thin layer chromatography where the solvent runs up the silica gel plate in an uneven fashion.

Figure 2 compares that thin layer chromatography results for the bacteria grown in a shaken medium versus a static medium. Shaken media better represents the environment that *V. fischeri* grows since the bacteria is found in saltwater and aquatic creatures—an environment that is filled with constant movement. However, a comparison between shaken versus static conditions was done in an attempt to achieve optimal results. The shaken environment could have possibly been a stressor to the bacteria, so a static medium could have provided different results for the bacteria. However, by observing Figure 2, it can be seen that the results of both the presence and relative quantities of the phospholipids did not change between shaken and static media. Once again, all four strains—WT, E1, A8, 78A—showed a presence of both PG and PE with PG in the largest amount while only WT and E1 showed evidence of producing CL.

Figure 3 compares the thin layer chromatography plate results for *V. fischeri* grown at room temperature—the traditional temperature most bacteria is grown—and at 15 ºC. The default temperature that the bacteria was grown at was room temperature, or 23 ºC. The contrasting condition for temperature was chosen as 15 ºC in order to, once again, better mimic the aquatic conditions that *V. fischeri* is found in since the average temperature of the oceans at the surface is approximately 17 ºC. However, an analysis of the thin layer chromatography done for the two temperatures as seen in Figure 3 shows the same trend displayed by both Figure 1

and Figure 2. There are no significant differences between the phospholipids found in bacteria grown at room temperature or 15 ºC. It can be seen that all four *V. fischeri* strains show presence of both PG and PE while only WT and E1 display signs of cardiolipin being produced. In Figure 3, the spots produced by thin layer chromatography seem to differ from the plates pictured in Figure 1 and Figure 2. The relative straight nature of the spots was a result of trouble-shooting in the procedures where a piece of filter paper soaked in the solvent was placed alongside the silica gel plate in the chromatography chamber. The filter paper provided a more even exposure of the solvent to the phospholipids present, allowing for a constant flow and therefore straight spots.

Conclusion

Discussion

The results provided from the experiment were both expected yet unexpected. It had been predicted that WT, E1, and A8 would display some presence of cardiolipin since these strains were chosen for having at least one gene that could have possibly produced cardiolipin. The strain 78A was predicted to not show any cardiolipin presence with thin layer chromatography since the genes associated with cardiolipin were removed. From the results in all three figures, it can be seen that the hypothesis regarding WT, E1, and 78A was proven correct. E1 showing the presence of cardiolipin means that the gene that was removed in this strain—*ymdC*, which has the highest homology with *Helicobacter pylori clsH* (cardiolipin synthase) of any *V. fischeri* protein—is not responsible for the production of cardiolipin in *V. fischeri*. In fact, the presence of cardiolipin in the strain E1 reinforced that *cls*, the gene that has been previously identified by scientists as a cardiolipin synthase, is the only gene responsible for producing cardiolipin in *V. fischeri*. The hypothesis regarding the strain A8/22A was proven incorrect by the results in all three figures. The strain A8 had a removal of the gene *cls* and kept the gene *ymdC*; by not displaying any presence of cardiolipin, the results of the strain A8 helps conclude that the gene *cls* is correct in being identified as a cardiolipin synthase and the protein encoded by the gene *ymdC* does not synthesize cardiolipin.

The purpose of the experiment, to analyze the phospholipids present in *V. fischeri*, was backed by previous studies regarding the flagellar sheaths and motility of both *Helicobacter pylori* and *V. fischeri*. Thin layer chromatography was performed regarding *V. fischeri* because *H. pylori* did not retain both its flagellar sheaths and motor function when cardiolipin-producing genes were removed, yet *V. fischeri* did retain both its flagella and motility when possible genes

regarding the production of cardiolipin were removed. The relationship between cardiolipin and flagellar sheaths is currently being studied at the University of Georgia, and the current hypothesis had been that cardiolipin is essential for the construction of flagellar sheaths. If the results of the thin layer chromatography for the strain 78A—which had the two possible cardiolipin-producing genes removed—had still displayed the presence of cardiolipin, it would suggest that there was an additional cardiolipin-producing gene that was not identified and the university's hypothesis was possibly correct. However, since strain 78A did not display cardiolipin and this *V. fischeri* strain still contained flagellar sheaths and retained motor function, the hypothesis that cardiolipin is necessary for flagella is possibly incorrect.

However, there are possible limitations to the conclusions provided by the results. The first limitation is the possibility of human error over the course of the experiment in the lab. It is possible that the extraction of lipids by the method of Bligh & Dyer was not completely successful due to the particular nature of the procedure. Another case of human error culd have occurred with the thin layer chromatography process in which the solvent was not allowed to run for a sufficient time or the staining reagent was unable to identify all the cardiolipin present in the four strains. An additional limitation is the unknown confounding variables in the lab environment; there could have been contaminating materials that could have been a stressor to the bacterial strains, inhibiting proper growth and possible cardiolipin production. The final limitation to the conclusion reached could be the relationship between cardiolipin and phosphatidylglycerol. Studies have shown that in the absence of cardiolipin, "the anionic phospholipid [phosphatidylglycerol], which shares the same glycerol head group as cardiolipin" has performed the same function of cardiolipin, especially in terms of polar localization which is where flagellar sheaths are produced (Rossi, Yum, Agaisse, & Payne, 2017). This is a possibility

since all three figures had displayed relatively large quantities of PG in all four strains. There is a possibility that phosphatidylglycerol took control of the same functions that cardiolipin has in regards to flagellar sheaths, leading to *V. fischeri* retained motility.

Further Research and Implications

The results and conclusions of this experiment has opportunities for further research and future implications. Since phosphatidylglycerol was found in high concentrations, reproducing this experiment with the removal of phosphatidylglycerol-producing genes can increase research about flagellar sheaths and bacteria mobility. The reproduction of this experiment in regards to phosphatidylglycerol can increase the scientific body of knowledge regarding the relationship between the various phospholipids. The implications of this experiment are broad due to the relative lack of knowledge about cardiolipin. The lack of information for this phospholipid and that the functions of cardiolipin are mostly hypothesized is significant since cardiolipin is virtually found in almost all organisms. Cardiolipin has been linked to cardiovascular failures, HIV 1, and mitochondrial dysfunctions (Paradies, Petrosillo, & Ruggiero, 2009). For instance, decreased cardiolipin levels and change in its acyl chain composition have been reported in heart failure and fatty liver disease (Petrosillo et al., 2007). The implications and further research opportunities surrounding phospholipids, especially cardiolipin, are endless.

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AP® RESEARCH
2018 SCORING COMMENTARY

Academic Paper

Sample: D
Score: 4

The paper earned a score of 4 because it presents a narrow topic of inquiry with an explicit connection to prior work in the study of these bacteria (page 3, paragraph 1, and page 4, paragraph 1 in which *V. fischeri* is rationalized as an alternative to *H. pylori*). The replicable research method is described in detail (pages 8–12), which is used to address the identified gap on page 4 (before the literature review), and limitations of the method are explained in the conclusion (pages 17 - 19). The implications are explained via connection to existing diseases as well as possible avenues for future research on page 19, paragraph 2.

The paper did not score a 3 because it describes its method clearly and identifies a distinct research gap that it then investigates through its research process.

The paper did not score a 5 because it neglects to tailor its use of language for the nonspecialist; as a result, its communication to the reader detracts from its overall impact. Finally, its discussion for implications for future research (page 19) is not as fulsome as one might expect given the complexity of the research project to that point (including the hyperbolic statement: “The implications and further research opportunities ... are endless”).