Effects of Fatty Acids in Bile on the Phospholipids of *Vibrio cholerae*

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Abstract

Enteric bacteria, such as *E. coli* and *V. cholerae,* encounter the digestive secretion bile in the small intestine. Bile is composed of mainly bile acids, lecithin, bilirubin, bicarbonate ions and fatty acids. Since bile possesses detergent like activity, bacteria have evolved mechanisms to avoid its bactericidal effect. *V. cholerae* senses bile and activates virulence genes that mediate motility, chemotaxis and adherence in preparation for colonization. The present study identifies a difference in the phospholipid profile of *V. cholerae,* but not *E. coli,* when grown in the presence and absence of bile. Mass spectrometry analysis of crude bile showed a variety of fatty acids including several long-chain and polyunsaturated fatty acids. To determine if the fatty acids in bile could be responsible for the differences in the phospholipids, *E. coli* and *V. cholerae* were grown in the presence of the individual fatty acids prior to phospholipid analysis. Growth of *E. coli* in fatty acids showed no significant changes in any phospholipids. However, growth of *V. cholerae* in fatty acids resulted in an upward shift of cardiolipin in longer chained fatty acids as well as the appearance of two species of phosphatidylethanolamine. There were also fatty acids that caused an appearance of lyso-phosphatidylethanolamine. These results demonstrate that *V. cholerae* can utilize a wider range of fatty acids than *E. coli* as reflected in membrane phospholipids. Specific long chain and polyunsaturated fatty acids are identified as being incorporated into *V. cholerae* phospholipids. *V. cholerae* is also associated with fatty acid rich marine environments. These environments include colonization of zooplankton and insect egg masses, as well as surviving in aquatic sediment. The survival advantage that this adaptation may provide is discussed.

1. Introduction:

When enteric bacteria such as *Vibrio cholerae* and *Escherichia coli* enter a host, they must encounter environmental stresses such as bile before they can cause an infection. The detergent-like properties of bile components such as bile acids and fatty acids are responsible for the bactericidal effect of bile. The way bacteria react to bile determines whether they can survive.

E. coli and *V. cholerae* have been studied to understand the bacterial response to bile. However the effect of bile on the bacterial phospholipids has not. This study concentrates on how the fatty acid content of bile affects the phospholipid profile of *E. coli* and *V. cholerae*.

1.1. *Vibrio cholerae*

Vibrio cholerae is an enteric pathogen that causes cholera, a diarrheal disease that is still responsible for outbreaks throughout the world, specifically in developing countries. Cholera is spread mostly through infected water and food and outbreaks are caused by poor sanitation in densely populated areas (Reidl and Klose). The disease causes a watery diarrhea and has symptoms of abdominal cramps, fever with vomiting and appearance of blood and mucus in stool (Senderovich *et al*.). However, this disease has a high infectious dose. There must be between 10^6 to 10^{11} colony forming units ingested in order contract the disease (Reidl and Klose).

Vibrio cholerae is a motile, Gram-negative bacterium that is classified as a curved rod. The three serotypes of *V. cholerae* that cause cholera infections are the classical 0395 and the El tor 01 and 0139 serotypes. However, there are about 200 other serotypes

of *V. cholerae* that are not associated with severe disease. In addition to causing disease, *Vibrio* species have natural aquatic habitats. They can attach to plants, green algae, copepods, crustaceans and insects in this habitat (Reidl and Klose). There are also various species that have been found to inhabit the digestive tract of various fish. The bacteria can be spread throughout the world by birds during their migration when the bacteria are bound to copepods and chironomids (Senderovich *et al.*).

1.2. *Escherichia coli*

Escherichia coli (E. coli) also causes diarrhea and is a major bacterium responsible for traveler's diarrhea. The bacteria are spread through contaminated food and water and infect the host's intestine (Willey *et al.*). *E. coli* is a Gram-negative enteric pathogen similar to *V. cholerae* (Merritt and Donaldson). There are many different strains of *E. coli* and most are nonpathogenic and are part of the normal flora. However, there are also some strains that do cause diarrhea which are classified by how the bacterium interacts with the host's epithelial cells in the intestine (Welley *et al.*). *E. coli* has been an important model system for studying lipid metabolism and biochemistry for the past 40 years. *E. coli*, along with other enteric bacteria, has evolved multiple mechanisms to sense and respond to intestinal bile. For example, *E. coli* withstands the toxic detergent effect of bile through active efflux of bile salts (Thanassi *et al.*). Furthermore, it was recently discovered that *E. coli* can sense the decrease in bile concentration. This allows *E. coli* to subsequently activate iron uptake machinery in preparation of colonizing the large intestine (Society for General Microbiology).

1.3. Bile

Vibrio cholerae are able to colonize the epithelial cells of the small intestine. The small intestine contains environmental stresses such as bile salts and organic acids (Reidl and Klose). Bile acts as a digestive secretion that works to disperse and absorb fats. The concentrations of bile acids are between .2 and 2% in the intestine (Gunn). Bile is a detergent mainly because bile acids are ampipathic molecules that break down other molecules or cells. Bile is able to enter cells by passing through the outer membrane or through porins in the membrane. This antibacterial property of bile causes a problem for the enteric bacteria in the intestine so the natural flora and enteric pathogens have mechanisms to resist bile (Gunn).

In order for bacteria to resist bile, they need to be able to sense it. However, the mechanisms by which bacteria sense bile are not completely understood. *E. coli* are believed to sense bile because of the bile induced damage to the membrane of the cell (Gunn). *E. coli* responds to this damage by using an efflux pump to remove the bile from the cell. One efflux pump in *E. coli* is from *acrAB* genes. These genes are regulated by *marRAB* operon resulting in overexpression of the AcrA/B efflux pump. During this overexpression, *E. coli* has higher resistance to bile as well as other antibiotics (Gunn). In addition to increasing expression of efflux pumps, *marRAB* also decreases expression of OmpF which decreases the amount of porins in the membrane to prevent bile or other substances from entering the cell (Prouty *et al*.).

V. cholerae has been identified to have multiple efflux pump systems to help avoid membrane damage from bile acids. These include the BreA/B, VceB VexB and VexD efflux pumps (Cerda-Maira *et al*.; Colmer *et al*.; and Bina *et al*.). It has been

shown that the ToxR-ToxT system is responsible for the bile resistance in *V. cholerae* (Gunn). ToxT may interact with a bile component and ToxR may regulate the increased bile resistance response (Provenzano *et al*.). ToxT is an activator of cholera toxin (CT) and the toxin coregulated pilus (TCP) (Schuhmacer and Klose). These virulence determinants are responsible in first causing colonization of the intestine (TCP) and then toxin-mediated cellular dehydration (CT). This results in watery diarrhea, or so-called rice water stool, associated with cholera. Upon exposure to bile, these virulence factors are reduced whereas motility is activated (Reidl and Klose; Gunn). It has been proposed that *V. cholerae* must migrate with its increased motility through the mucin layer of the small intestine in order to colonize the epithelial cells which have a lower bile concentration, where it is then able to induce virulence factors (Reidl and Klose). ToxR regulates OmpU and OmpT which are porins for the outer membrane. When *V. cholerae* is grown in bile, a porin more permeable to the negatively charged molecules like bile salts, OmpT, is downregulated and the less permeable porin, OmpU, is upregulated resulting in higher resistance to bile (Gunn; Reidl and Klose). This suggests that *V. cholerae* changes its outer membrane proteins to resist bile.

1.4. Exogenous Fatty Acid Utilization

One of the components of bile is fatty acids. In bacterial cells, exogenous fatty acids can represent an important carbon source as well as substrate for fatty acid and phospholipid synthesis. The structural characteristics of fatty acids can also affect membrane permeability (Wydro). In order for fatty acids to be utilized, they need a way to enter the cell so they must pass through the bacterial membranes. Gram-negative

bacteria such as *E. coli* and *V. cholerae* possess two membrane bilayers known as the inner membrane and the outer membrane. The inner membrane consists of phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin. The outer membrane consists of an outer leaflet of lipopolysaccharide and an inner leaflet of phosphatidylethanolamine (Wydro). In *E. coli*, the membrane normally has a phospholipid makeup of 75% phosphatidylethanolamine, 20% phosphatidylglycerol and 5% cardiolipin while *V. cholerae* has 60 to 80% phosphatidylethanolamine and 15 to 30% phosphatidylglycerol (Oliver and Colwell). Nutrients are able to enter Gram-negative bacterial cells through outer membrane proteins that are nonspecific porins, substrate specific porins or high affinity substrate specific transport proteins (DiRusso and Black).

It was shown in *E. coli* that fatty acids are transported into the cell through the gene product of *fadL* (Han *et al*.). FadL is an outer membrane protein that is a substrate specific channel and regulates fatty acid intake through environmental factors such as osmolarity (Higashitani *et al*.; DiRusso and Black). Between the outer and inner membrane, there is periplasmic space in which a tail-specific protease has been implicated in transporting fatty acids (DiRusso and Black). The inner membrane contains fatty acyl CoA synthase (FACS) from the FadD gene which activates the fatty acid components to CoA thioesters (DiRusso and Black; Byers). This process works through the use of ATP and a proton gradient of the membrane (DiRusso and Black*). V. cholerae* contains proteins for the transport and utilization of fatty acids that are similar to FadL and FadD (Byers). There is also evidence of a cotransporter of fatty acids and protons in the inner membrane which involves an oleic acid binding protein (Kameda; Kameda *et*

al.). The intake of fatty acids is both specific and tightly regulated by the bacterial cells. If the levels of fatty acids become too high, then transcription is of both *fadL* and *fadD* is decreased (DiRusso and Black).

In *E. coli*, there are two different pathways for utilization of fatty acids. There is the exogenous fatty acid pathway that uses coenzyme A and there is the synthesized fatty acid pathway that uses the fatty acyl-acyl carrier protein intermediates. These two pathways have chemically distinct acyl intermediates that are able to distinguish between exogenous and synthesized fatty acids (Byers). Recently however, proteins called acyltransferases were discovered to convert both the intermediates acyl-CoA from exogenous fatty acids and acyl-ACP from synthesized fatty acids into precursors of phospholipids. This means that exogenous fatty acids can be used to synthesize phospholipids for the membrane instead of relying only on the synthesis pathway which requires more energy (Zhang and Rock, 2008a). When bacteria encounter environmental stresses, such as solvent stress, they modify the fatty acid profile of the phospholipids of the cytoplasmic membrane. The baceria will increase both the fatty acid saturations and the average chain length of the fatty acids. This stabilizes the bilayer and enhances rigidity in order to have an increase tolerance to organic solvents (Morotomi *et al*.).

1.5. Environmentally and Physiologically Relevant Fatty Acids

Both long chain and polyunsaturated fatty acids are known to be present in the various environments inhabited by *V. cholerae*. Omega 3 polyunsaturated fatty acids (PUFAs) are essential dietary needs (Giamareelos). PUFAs have been shown to affect gastrointestinal microbiota's attachment through possibly creating a different fatty acid

profile for the intestinal wall (Bomba *et al*.). The major PUFAs in humans are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are found in several species of bacteria that have marine habitats (Allen and Bartlett; Metz *et al*.). EPA and DHA in the membrane help to maintain membrane fluidity as acyl components of membranes (Okuyama *et al*.). These fatty acids can make up to 30% of the total fatty acids found in the phospholipids of marine psychrophilic and piezophilic bacteria (Okuyama *et al*.). Longer chained polyunsaturated fatty acids create a more hydrophobic environment which causes harder entry into bacterial cells (Nishida *et al*.). This could impede the uptake of nutrients into the cell (Maczulak *et al*.). In addition to being found in seafood, the long chain omega 3 fatty acids are distributed from marine seaweed and microalgae (Calder and Yaqoob, Okuyama *et al*.). They can also be found in macroalgae and marine crustaceans such as copepods, which are known to be an environmental reservoir of *V. cholerae* (Bhaskar *et al*., Jeffs *et al*.).

Long chain fatty acids (LCFAs), defined as fatty acids with an aliphatic tail longer than 12 carbons, have been shown to be present in bile and in marine environments such as algae (Chatterjee *et al*.; Zheng *et al*.). Relevant LCFAs such as γ-linolenic acid (GLA) and arachidonic acid (AA) along with DHA were found to interact with *E. coli* by inhibiting growth or selecting for strains that were resistant to β-lactams. This could happen by becoming incorporated into the cell wall or by their peroxidation (Giamarellos-Bourboulis *et al*.). It has been shown that *V. cholerae,* like other bacteria, is more sensitive to unsaturated fatty acids that to saturated fatty acids (Chatterjee *et al*.). LCFAs and PUFAs are environmentally and physiologically relevant for *V. cholerae* because they are present in both the aquatic and host environment.

This study aims to research the effects of exogenous fatty acids present in bile on the phospholipid profiles of *E. coli* and *V. cholerae*. The results show that *V. cholerae* is able to uptake and utilize more fatty acids than *E. coli*. This may indicate an adaptation in *V. cholerae* used for surviving in both the host and aquatic environments.

2. Materials and Methods:

2.1. Bacterial Strains and growth conditions

The *E. coli* K-12 laboratory strain BW25113, the parent strain of the Keio collection, and the *V. cholerae* El tor strain C6706 (Peru isolate) were used in this study. The strains were selected from a single colony from plated bacteria and grown overnight in a culture of Luria broth. The cultures were then diluted to an optical density of 0.05 and grown in 20 ml cultures of Luria broth at 37°C in a shaking incubator. The cultures were grown with loose caps for aeration. In some cultures, bile (0.4%) or individual fatty acids (300 or 500 µM) were added to the 20 ml cultures prior to inoculation. Particular notice was taken as to whether the fatty acids fell out of the solution when they were added. This was not observed in any culture to any significant extent.

2.2. Mass Spectrometry

Normal phase LC-ESI/MS of lipids was performed using an Agilent 1200 Quaternary LC system coupled to a QSTAR XL quadrupole time-of-flight tandem mass spectrometer (Applied Biosystems, Foster City, CA). An Ascentis® Si HPLC column (5 µm, 25 cm × 2.1 mm) was used. Mobile phase A consisted of chloroform/methanol/aqueous ammonium hydroxide (800:195:5, v/v/v). Mobile phase B

consisted of chloroform/methanol/water/aqueous ammonium hydroxide (600:340:50:5, v/v/v/v). Mobile phase C consisted of chloroform/methanol/water/aqueous ammonium hydroxide (450:450:95:5, v/v/v/v). The elution program consisted of the following: 100% mobile phase A was held isocratically for 2 min and then linearly increased to 100% mobile phase B over 14 min and held at 100% B for 11 min. The LC gradient was then changed to 100% mobile phase C over 3 min and held at 100% C for 3 min, and finally returned to 100% A over 0.5 min and held at 100% A for 5 min. The total LC flow rate was 300 μl/min. The post-column splitter diverted ~10% of the LC flow to the ESI source of the Q-Star XL mass spectrometer, with MS settings as follows: IS = -4500 V, CUR = 20 psi, GS1 = 20 psi, DP = -55 V, and FP = -150 V. Nitrogen was used as the collision gas for MS/MS experiments. Data acquisition and analysis were performed using the instrument's Analyst QS software.

2.3. Bile and Fatty Acids

Bovine bile, obtained from Sigma, was added to the Luria broth at a final concentration of 0.4% which is approximate to the percentage in the lumen of the intestine where *V. cholerae* first encounters it. The bile was prepared by weighing 0.2 grams of crude bovine bile and adding it to 50ml of Luria broth. The solution was then autoclaved and cooled before aliquoting 20ml per tube and inoculating the cultures.

Individual fatty acids were obtained commercially. Palmitic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid, γ-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid were obtained from Sigma. Dihomo-γ-linolenic acid and α-linolenic acid were obtained from Cayman Chemicals. Table 1

shows the fatty acids used in this study and how they were prepared. Once solubilized, all fatty acids were either immediately used or stored at 20°C. Heptadecanoic was used at 300 µM because bacterial growth was inhibited at 500 µM. In order to show that there were no changes because of the solvents or chemical used to dissolve the fatty acids, controls were run with Luria broth and 333 µl of ethanol, 61 µl methanol, 20 µl chloroform and 42mg of Brij 58 determined by the largest amount used in the cultures.

2.4. Isolation of Phospholipids

The cultures were grown to log phase (OD = 0.8-1.0) and then transferred to smaller tubes and allowed to pellet in a centrifuge at 4250 rpm for 10 minutes. The Luria broth was poured off and the cells were washed with 5 ml of phosphate buffered saline and vortexed. Following another 10 minute centrifugation, the phosphate buffered saline was poured off and the pelleted cells were either stored at -20°C or were processed through the method of Bligh and Dyer (Bligh and Dyer).

The Bligh-Dyer method was used to isolate the phospholipids from the pelleted cells. 5 ml of 1:2:0.8 of chloroform, methanol and water, respectively, was added to the pelleted cells for lysis. The solution was vortexed and allowed to sit for 20 minutes. Then the solution was centrifuged for 10 minutes at 2100g. The supernatant containing phospholipids was removed from the pelleted cells and transferred to another tube. Then a two-phase solution was made by adding 1.3 ml of chloroform and 1.3 ml of water into the tubes. After vortexing, the tubes were centrifuged for another 10 minutes and the lower phase was removed and added to a new tube. 2.6 ml of chloroform was added into the supernatant and the tube was vortexed and centrifuged another 10 minutes. The lower

phase was again extracted and added to the tube containing the previous lower phase. The lipids were washed by adding 5.2 ml methanol and 3.6 ml of water. Following centrifugation, the final extraction contained in the combined lower phases, was dried down under a stream of nitrogen.

2.5. Thin Layer Chromatography

The dried phospholipids obtained from the Bligh-Dyer method were then used to spot onto a TLC plate. The weight of each sample was determined by resuspending in 4:1 chloroform to methanol transferring into preweighed 1.5 ml micro V vials (National Scientific). Equal weights of each sample (approximately 0.4 mg) were spotted onto silica coated glass plates (TLC) from EMD chemicals and run in a 65:25:10 (chloroform:methanol:acetic acid) tank for 2 hours and 10 minutes to separate the lipids by thin layer chromatography. After drying the plates, they were sprayed with 10% sulfuric acid in 100% ethanol, which oxidizes the phospholipids prior to charring on a hot plate at 200°C to visualize carbon containing species. These plates were cooled and then imaged with a Canon scanner (CanoScan 8600F).

3. Results:

3.1. Bile exposure results in an altered phospholipid profile for *V. cholerae* but not *E. coli*

When grown in the presence of bile, the phospholipid profile of *E. coli* remains the same while the phospholipids of *V. cholerae* display several changes (Figure 1). For *V. cholerae* exposed to bile, the cardiolipin shifts up meaning there is a more hydrophobic species of cardiolipin. Also, lyso-phosphatidylethanolamine appears in the

phospholipid profile when *V. cholerae* is exposed to bile. In addition, an unknown phospholipid appears beneath the lyso-phosphatidylethanolamine. There is also a decrease in phosphatidylethanolamine and phosphatidylglycerol during this exposure. However, when *E. coli* is exposed to bile, charring does not show any difference between the phospholipid profiles (Figure 1).

3.2. Crude bile contains a variety of fatty acids, many that are long-chain and polyunsaturated

In order to find out what in bile could be affecting the phospholipid profile of *V. cholerae*, the phospholipids of crude bile were isolated from the method of Bligh and Dyer (Bligh and Dyer). This lipid fraction was sent for mass spectrometry analysis. The analysis showed a variety of fatty acids that were both long chain and polyunsaturated (Figure 2A). The fatty acids shown to be present were 14:0, 16:0, 16:1, 17:0, 18:1, 18:2, 20:3, and 20:4. These correspond to palmitic, heptadecanoic, stearic, oleic, linoleic, arachidonic, and dihomo-γ-linolenic acids, respectively.

3.3. *V. cholerae* grown in bile exhibits an altered fatty acid composition

V. cholerae was grown with and without bile and the isolated fatty acids were also analyzed with mass spectrometry. Figure 2B shows that when *V. cholerae* is grown in bile, multiple fatty acids are obtained that were not detected in the absence of bile. In addition to the fatty acids found in bile alone, this analysis also detected 18:3, 20:5 and 22:6 fatty acids. These fatty acids correspond to α and/or γ-linolenic, eicosapentaenoic and docosahexaenoic acid.

3.4. Solubilization of individual fatty acids did not affect the phospholipid profiles of *E. coli* or *V. cholerae*

As described in the Materials and Methods section, the commercially acquired fatty acids were solubilized in chloroform, methanol, ethanol or Brij 58 with ethanol. To test whether the solvents affected the phospholipid profile by themselves, the bacteria were grown in each individual solvent. Subsequently, the lipids were isolated and analyzed. When grown with these solutions containing no fatty acids solubilized, there were no shifts in the phospholipids in either *E. coli* or *V. cholerae* (Figure 3). The relative ratios of the major phospholipids remained the same. Although not shown, the solution that contains 1% TWEEN in water diluted in 0.1M sodium phosphate buffer also had no effect on phospholipid profiles of either bacteria. Thus, it was determined that the solvents, at the concentrations used in this study, do not alter the phospholipid profiles of *E. coli* and *V. cholerae.* A complete list of the fatty acids and their structures used in this study is shown in Figure 4.

3.5. *E. coli* shows little change in phospholipids when grown in the presence of individual fatty acids from bile

When grown in the presence of the fatty acids determined from the mass spectrometry of bile, there is little change in the phospholipid profile of *E. coli* (Figure 5). There is a slight upward shift in cardiolipin in oleic and linoleic acids, but this is expected based on *E. coli's* FadL-FadD machinery's substrate preference (Black). Besides these two fatty acids, there were no other observed shifts in cardiolipin due to growth with fatty

acids. There were not any apparent shifts of the phospholipids phosphatidylglycerol or phosphatidylethanolamine due to any of the fatty acids that *E. coli* was grown in, nor did any new phospholipid species appear.

3.6. *V. cholerae* shows changes in its phospholipids when grown in the presence of individual fatty acids from bile

When grown in the presence of fatty acids determined to be in bile, there were many changes in the phospholipid profiles of *V. cholerae* (Figure 6). In *V. cholerae* grown in the presence of palmitic acid, heptadecanoic acid and stearic acid, there were no shifts in phospholipids. In fact, growth in heptadecanoic acid caused a slight downshift in phospholipids. There was also an unknown phospholipid seen in the presence of bile that appeared during growth with heptadecanoic acid. When grown in the presence of oleic and linoleic acid, cardiolipin showed the same upward shift that was seen in *E. coli* while there was no change in any other phospholipids. Growth in the presences of α-linolenic acid causes an upward shift in cardiolipin that gradually increases as *V. cholerae* is grown in fatty acids with increasing length from γ-linolenic to dihomo-γ-linolenic, arachidonic, eicosapentaenoic and finally to docosahexaenoic acid. The major phospholipids phosphatidylethanolamine, phosphatidylglycerol and cardiolipin become more hydrophobic species as the exogenous fatty acid increased in length. For phosphatidylglycerol and phosphatidylethanolamine, The effect of docosahexaenoic acid was better observed by spotting a lower amount (0.2 mg) of *V. cholerae* phospholipids (Figure 7). *V. cholerae* produces a higher phospholipid in response to the exogenous fatty

acids while maintaining the lower species. In growth with α-linolenic acid, lyso-phosphatidylethanolamine and the same unknown phospholipid appear.

4. Discussion

This study identifies a difference in the phospholipid profile of *V. cholerae* when grown in bile compared to *E. coli's* which did not change. Crude bile and *V. cholerae* grown in the presence of bile were shown to have the following fatty acids: palmitic, heptadecanoic, stearic, oleic, linoleic, α and/or γ-linolenic, arachidonic, dihomo-γ-linolenic eicosapentaenoic and docosahexaenoic. When grown in the presence of these individual fatty acids, *E. coli* showed little change in its phospholipid profile. However, when *V. cholerae* was grown in these fatty acids, its phospholipid profile reflected that it was able to incorporate long chain and polyunsaturated acids into its membrane.

The shifts in cardiolipin during bile exposure is likely due to the incorporation of exogenous fatty acids within bile. It is easier to recognize by thin layer chromatography because it contains four acyl chains whereas phosphatidylglycerol and phosphatidylethanolamine only contain two acyl chains. The exogenous fatty acids in bile are also assumed to incorporate into phosphatidylglycerol and phosphatidylethanolamine. However, their differential migration cannot be observed in the selected thin layer chromatography system.

The *E. coli* genes involved in uptake of exogenous fatty acids are FadL and FadD (Byers). By searching the Clusters of Orthologous Groups (COG) database of these two genes in *E. coli* and then performing a Basic Level Alignment Search Tool (BLAST) search on the National Center for Biotechnology Information website, multiple homologs

were revealed in *V. cholerae*. There were three homologs in *V. cholerae* for both the *E. coli* FadL and FadD genes. Since the homologs are present in even numbers, it is a possibility that these genes could be paired up such that they can accommodate different fatty acids. Also, like *E. coli*, the FadL and FadD homolog genes do not appear in the same operon suggesting difference in regulation of gene expression. Furthermore, *V. cholerae* also have homologs for all of the known acyltransferases in both Gram-positive and Gram-negative bacteria. It also has 2 homologs of *plsC*. PlsC an acyltransferase that is now to utilize acyl-ACP or acyl-CoA to add fatty acids to membrane phospholipids (Zhang and Rock, 2008a). Thus, *V. cholerae* has many inner membrane proteins that might contribute to utilizing several different fatty acids. In *E. coli,* the FadL and FadD machinery have an optimized binding affinity for oleic (18:1) and palmitic (16:0) acids (Black). The extra sets of FadL and FadD genes in *V. cholerae* may be optimized to also take up other fatty acids such as a set that preferentially takes up 20 and 22 carbon fatty acids.

V. cholerae being able to uptake and utilize many fatty acids could be an advantage that aids in its survival. Because fatty acids can be used as a carbon source, *V. cholerae* would be able to survive in environments that contain limited nutrients if they have fatty acids available. Examples of these types of environments are marine environments. Oceans contain a variety of fatty acids such as lauric, myristic, palmitic, oleic, linoleic and linolenic acids in differing amounts depending on the depth (Slowey *et al.*). Sediment, another environmental reservoir of *V. cholerae,* also contains long chain fatty acids of 20 to 30 carbons (Wakeham *et al*.). Other environmental reservoirs of *Vibrio* species expected to contain large amounts of palmitic, oleic, α or γ-linoleic,

eicosapentaenoic or docosahexaenoic acids include estuaries, copepods and chironomid egg masses (Dumontet *et al*.; Jeffs *et al*.; Halpern *et al*.). *V. cholerae* is known to attach to copepods whereas *E. coli* does not adhere to the crustaceans (Dumontet *et al*.). There is also an increase in the concentration of lipids in the summer which coincides with an increase of copepods (Jeffrey; Jeffs *et al*.). It is speculated that because of these occurrences, especially when the temperature rises, *V. cholerae* comes out of the sediment into the water environments (Dumontet *et al*.). This takes places around the same time that cholera outbreaks usually occur. These environments all contain fatty acids that were used in this study and were found relevant because of their presence in bile. While it is unknown what fatty acids are available from copepods, the presence of a variety of fatty acids coupled with *V. cholerae*'s ability to utilize them would give *V. cholerae* an advantage for survival in these environments.

Polyunsaturated fatty acids are essential dietary needs that play roles in cell signaling, gene expression, and in cardiovascular health and can be found in omega-3s and omega-6s (Calder and Yaqoob). These can be found in many dietary sources including fish, flaxseed, walnuts, eggs, meat, milk, cheese, algae and some fruits and vegetables such as strawberries or broccoli. The same way *V. cholerae*'s ability to uptake and utilize fatty acids in nutrient-limited marine environments may aid in survival, survival in the host may be helped by the ability to uptake and utilized fatty acids present in substances like bile or from the hosts diet. Dietary intake has also been shown to affect symbiotic relationships. Transcription in the symbiotic relationship between *Vibrio fischeri* and the squid has been proven to go through at least two distinct metabolic states that are directly due to nutrition of the host (Wier *et al*.). The same study showed that the

fatty acid profile of *V. fischeri* changed significantly due to the exogenous fatty acids available in the squid's light emitting crypt (Wier *et al*.). It has also been shown that the lipids obtained through dietary intake influence the gastrointestinal normal flora. A diet high in PUFAs can encourage growth of specific bacteria that are present compared to diets that don't contain increased amounts of PUFAs. This may be because the fatty acid intake modifies the composition of fatty acids in the intestinal walls so that attachment for normal flora changes (Bomba *et al*.). An example of how this may affect infection by *V. cholerae* is that many cholera infections come from contaminated seafood which are rich in PUFAs and could then cause a change in the gastrointestinal environment and possibly aid in survival and/or virulence of cholera within the host.

V. cholerae is known to produce a secreted lecithinase that can cleave phosphatidylcholine. This process would generate lyso-phosphatidylcholine and a free fatty acid. The phosphatidylcholine species in the human intestine contains 18:0, 18:1, and 18:2 fatty acids. These could all become available for uptake following lecithinase activity (Bruan *et al*.). It is unknown if the lecithinase is secreted during infection. However, it is possible that *V. cholerae* could break up the hydrophobic phosphatidylcholine barrier in mucin and utilize the products as a carbon source.

It is not clear why *V. cholerae* changes its phospholipids, but it may be to help the membrane during environmental stresses. However, it is unknown how the membrane of *V. cholerae* changes depending on the different fatty acids taken in. The membrane is comprised of saturated and unsaturated fatty acids. Saturated fatty acids are linear and cause the membrane to pack together. This causes a decrease in fluidity and in permeability of the membrane. Branched saturated fatty acids cause an increase in

fluidity because the membrane is not as packed together. Unsaturated fatty acids can also create more membrane fluidity by increasing the amount of *cis* bonds. By creating a *cis* bond, kinks are created in the acyl chains and the membrane becomes less ordered whereas *trans* bonds create more order because they do not cause kinks (Zhang and Rock, 2008b). The FabA protein β-hydroxydecanoyl dehydrase is the key enzyme during *de novo* fatty acid synthesis that creates a *cis* double bound into 10-carbon intermediates (Mansilla et al.).

V. cholerae also contain a *cis-trans* isomerase. This allows the fatty acids to be transformed between having *cis* and *trans* double bounds after the phospholipid is in the membrane. This is important in the adaptation of *V. cholerae* to environmental stresses because it allows the bacterium to change the fluidity and permeability of its membrane (Heipieper *et al.*). However, this also means that *V. cholerae* are able to change fatty acids between the two conformations so that you cannot assume that because the cell uptakes a fatty acid with a *cis* conformation that the fatty acid is still in the *cis* conformation when the fatty acids is actually utilized inside of the cell. This study did not analyze membrane permeability of the cell so it is unknown whether fatty acids have any affect. A better understanding of the permeability of the membrane could be obtained by using gas chromatography to determine the *cis/trans* conformation of the fatty acids in *V. cholerae*.

The membrane is mostly made up of the phospholipids phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin. Phosphatidylglycerol serves a very similar function in the bacterial membrane as cholesterol does in mammalian membranes. The more phosphatidylglycerol present, the more repulsive

forces towards each other because phosphatidylglycerol is negatively charged. This causes more fluidity in the membrane. Phosphatidylethanolamine with saturated acyl chains causes the membrane to be more packed and since it posses zwitterionic charteristics, phosphatidylethanolamine is able to decrease the amount of repulsive forces from phosphatidylglycerol and cause less fluidity (Wydro and Witkowska; Zhao *et al*.; Murzyn *et al*.). Cardiolipin has a large head group that is charged which allows tight binding with other molecules. For example, cardiolipin can stabilize areas of proteins like a clamp (Palsdottir and Hunte). Phospholipids are also able to go through the process of acyl chain tailoring which is important for adaptive responses in bacterial membranes such as in low temperatures. Examples of acyl chain tailoring are changing the rations between phospholipids containing 18:1/20:5 and 18:1/22:6. *Shewanella gelidimarina* is an example of a bacterium that can change the proportion between these fatty acids in its phospholipids (Nicholos *et al*.).

In the current study, qualitative analysis of the phospholipid profile of *V. cholerae* grown in the presence of bile shows an increase in the amount of cardiolipin, a decrease in the amount of phosphatidylglycerol and phosphatidylethanolamine and an increase in lyso-phosphatidylethanolamine. From data not shown, quantitative changes of these observations have been confirmed. Based on the phospholipid characteristics described above, these alterations would be expected to decrease overall fluidity of the membrane. With less permeability, the membrane would be expected to aid in survival within the host, especially when encountering bile or membrane-targeting immune effectors, such as antimicrobial peptides.

These phospholipids also have functions in the membrane pertaining to proteins. With tight interactions between the phospholipids and proteins, the phospholipids are important for folding the membrane proteins and integrating them into the membrane (Palsdottir and Hunte). Similarly, phosphatidylethanolamine is needed in order for the *E. coli* lactose permease (LacY) to be folded correctly (Wydro and Witkowska). Both phosphatidylethanolamine and phosphatidylglycerol are also needed for domain formation and protein clustering in the membrane (Wydro and Witkowska). The lipid bilayer causes the membrane proteins to preserve their structure in the membrane because the lipids cause a lateral pressure in the membrane (Palsdottir and Hunte). Cardiolipin has also been proposed to play a part in the proton (Cl/K) uptake pathway (Palsdottir and Hunte). It is possible that the phospholipid profile induced by bile contributes to virulence. The new phospholipid profile could be stabilizing the *V. cholerae* membrane proteins induced in virulence. However, this study only analyzed the phospholipids that were present in the cell after growth and not the functionality of proteins within the cell.

Chatterjee and Chowdhury tested the effects of bile on cholera toxin. They found that bile contained the saturated palmitic and stearic acid, and the unsaturated linoleic, oleic and arachidonic acids (Chatterjee and Chowdhury). They also found in another study that *V. cholerae* is more sensitive to unsaturated fatty acids than it is to saturated fatty acids (Chatterjee *et al*.). In this study, it was observed that not only were palmitic, stearic, linoleic, oleic and arachidonic acids present in samples of bile, but that heptadecanoic and dihomo-γ-linolenic acids were also present in bile samples. It was also found that *V. cholerae* grown in bile also contained the fatty acids α and/or γ-linolenic, eicosapentaenoic and docosahexaenoic acids. This study further showed that *V. cholerae*

uptakes the unsaturated fatty acids from bile, as well as fatty acid found in various *Vibrio* habitats, and utilizes these in its membrane causing a change in the phospholipid profile. The longer the carbon chain and the more unsaturated the fatty acids are, the higher the shift in the phospholipids phosphatidylglycerol, phosphatidylethanolamine and cardiolipin which emphasizes the adaptation of *V. cholerae* to utilize a variety of fatty acids and may impact its survival in many low nutrient environments such as the host.

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Work Cited

Allen, Eric E., and Douglas H. Bartlett. "Structure and regulation of the omega-3 polyunsaturated fatty acid synthase genes from the deep-sea bacterium Photobacterium profundum strain SS9." *Microbiology* 148 (2002): 1903-1913. http://mic.sgmjournals.org/cgi/content/abstract/148/6/1903 (accessed April 28, 2010).

Bhaskar, N, T Kinami, SB Park, Y Endo, and K Fujimoto. "Occurrence of conjugated polyenoic fatty acids in seaweeds from the Indian Ocean." Abstract, *Verlag der Zeitschrift für Naturforschung* 59, no. 5-6 (May 2004): 310-4. http://www.znaturforsch.com/ac/v59c/s59c0310.pdf (accessed April 28, 2010).

Bina, J. E., D. Provenzano, C. Wang, X. R. Bina, and J. J. Mekalanos. "Characterization of the *Vibrio cholerae* vexAB and vexCD efflux systems." *Arch. Microbiol.* 186 (2006): 171-181. doi:10.1007/s00203-006-0133-5 (accessed May 2, 2010).

Black, Paul N. "Characterization of FadL-specific fatty acid binding in *Escherichia coli*." Abstract, *Bichimica et Biophysica Acta* 1046 (1990): 97. doi:10.1016/0005-2760(90)90099-J (accessed April 26, 2010).

Bligh, E G, and W J Dyer. "A Rapid Method of Total Lipid Extraction and Purification." *Canadian Journal of Physiology and Pharmacology* 37, no. 8 (1959): 911-917. doi:10.1139/y59-099 (accessed May 4, 2010).

Bomba, A., R. Nemcova, S. Gancarcikova, R. Herich, P. Guba, and D. Mudronova. "Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids." *British Journal of Nutrition* 88 (2002): S95-S99. doi:10.1079/BJN2002634 (accessed April 26, 2010).

Braun, Annika, Irina Treede, Daniel Gotthardt, Anke Tietje, Alexandra Zahn, Rebecca Ruhwald, Ulrike Schoenfeld, Thilo Welsch, Peter Kienle, Gerhard Erben, and Wolf-Dieter Lehmann. "Alterations of phospholipid concentration and species composition of the intestinal mucus barrier in ulcerative colitis: a clue to pathogenesis." *Inflammatory Bowel Disorders* 15, no. 11 (November 2009): 1705-1720. doi:10.1002/ibd.20993 (accessed May 2, 2010).

Byers, David M. "Elongation of Exogenous Fatty Acids by the Bioluminescent Bacterium *Vibrio harveyi*." *Journal of Bacteriology* 171, no. 1 (January 1989): 59-64. doi:0021-9193/89/010059-06 (accessed April 28, 2010).

Calder, PC, and P. Yaqoob. "Understanding omega-3 polyunsaturated fatty acids." Abstract, *Postgraduate Medicine* 121, no. 6 (November 2009): 148-57. http://www.ncbi.nlm.nih.gov/pubmed/19940425 (accessed April 28, 2010).

Cerda-Maira, Francisca A., Carol S. Ringelberg, and Ronald K. Taylor. “The Bile Response Repressor of BreR Regulates Expression of the *Vibrio cholerae* breAB Efflux System Operon.” *Journal of Bacteriology* 190, no. 22 (November 2008): 7441-7452. doi:10.1128/JB.00584-08 (accessed May 2, 2010).

Chatterjee, Arpita, and Rukhsana Chowdhury. “Bile and Unsaturate Fatty Acids Inhibit the Binding of Cholera Toxin and *Escherichia coli* Heat-Labile Enterotoxin to GM1 Receptor.” *Antimicrobial Agents and Chemotherapy* 52, no. 1 (January 2008): 220-224. doi:10.1128/AAC.01009-07 (accessed April 17, 2010).

Chatterjee, Arpita, Pradeep K. Dutta, and Rukhsana Chowdhury. “Effect of Fatty Acids and Cholesterol Present in Bile on Expression of Virulence Factors and Motility of *Vibrio cholerae*.” *Infection and Immunity* 75, no. 4 (April 2007): 1946-1953. doi:10.1128/IAI.01435-06 (accessed April 28, 2010).

Colmer, J. A., J. A. Fralick, and A. N. Hamood. “Isolation and characterization of a putative multidrug resistance pump from *Vibrio cholerae* .” *Molecular Microbiology* 27, no. 1 (1998): 63-72. doi:10.1046/j.1365-2958.1998.00657.x (accessed May 2, 2010).

DiRusso, Concetta C., and Paul N. Black. “Long-chain fatty acid transport in bacteria and yeast. Paradigms for defining the mechanism underlyin the protein-mediated process.” *Molecular and Cellular Biochemistry* (1999): 41-52. doi:10.1023/A:1006823831984 (accessed April 17, 2010).

Dumontet, S., K. Krovacek, S. B. Baloda, R. Grottoli, V. Pasquale, and S. Vanucci. “Ecological Relationship Between *Aeromonas* and *Vibrio* spp. and Planktonic Copepods in the Coastal Marine Environment in Southern Italy.” *Comparative Immunology, Microbiology, and Infectious Diseases* 19, no. 3 (1996): 245-254. doi:10.1016/0147-9571(96)00012-4 (accessed April 26, 2010).

Giamarellos-Bourboulis, E. J., P. Grecka, A. Dionyssiou-Asteriou, and H. Giamarellou. “In vitro activity of polyunsaturated fatty acids on *Pseudomonas aeruginosa*: relationship to lipid peroxidation.” *Prostaglandins, Leukotrienes and Essential Fatty Acids* 58, no. 4 (1998): 283-287. doi:10.1016/S0952-3278(98)90037-0 (accessed April 28, 2010).

Gunn, John S. “Mechanisms of bacterial resistance and response to bile.” *Microbes and Infection*, no. 2 (2000): 907-913. doi:s1286457900003920/Rev (accessed April 8, 2010).

Halpern, Malka, Ori Landsberg, Dina Raats, and Eugene Rosenberg. “Culturable and VBNC *Vibrio cholerae*: Interactions with Chironmid Egg Masses and Their Bacterial Population.” *Microbial Ecology* 53 (2007): 285-293. doi:10.1007/s00248-006-9094-0 (accessed May 4, 2010).

Han, Mee-Jung, Jeong Wook Lee, Sang Yup Lee, and Jong Shin Yoo. “Proteome-Level Responses of Escherichia coli to Long-Chain Fatty Acids and Use of Fatty Acid Inducible Promoter in Protein Production.” *Journal of Biomedicine and Biotechnology* 2008, no. 735101 (2008): 12 pages. doi:10.1155/2008/735101 (accessed April 28, 2010).

Heipieper, HJ, F Meinhardt, and A Segura. “The cis-trans isomerase of unsaturated fatty acids in Pseudomonas and Vibrio: biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism.” *FEMS Microbiology Letters* 229, no. 1 (2003): 1-7. doi:10.1016/S0378-1097(03)00792-4 (accessed April 27, 2010).

Higashitani, A, Y Nishimura, H Hara, H Aiba, T Mizuno, and K Horiuchi. “Osmoregulation of the fatty acid receptor gene fadL in *Escherichia coli*.” Abstract, *Molecular and General Genetics* 240, no. 3 (September 1993): 339-47. doi:10.1007/BF00280384 (accessed April 28, 2010).

Jeffrey, Lela M. “Lipids in Sea Water.” *The Journal of the American Oil Chemists’ Society* 43, no. 4 (1966): 211-214. doi:10.1007/BF02641089 (accessed April 26, 2010).

Jeffs, Andrew G., Peter D. Nichols, Ben D. Mooney, Katrina L. Philips, and Charles F. Phleger. “Identifiying potential prey of the pelagic larvae of the spiny lobster *Jasus edwardsii* using signature lipids.” *Comparitive Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 137, no. 4 (April 2004): 487-507. doi:10.1016/j.cbpc.2004.02.003 (accessed April 26, 2010).

Kameda, K. “Partial purification and characterization of fatty acid binding proteins(s) in Escherichia coli membranes and reconstitution of fatty acid transport system.” *Biochem Int* 13 (1986): 343-350. (accessed May 5, 2010).

Kameda, K, LK Suzuki, and Y Imai. “Transport of fatty acid is obligatory coupled with H+ entry in pheroplasts of Escherichia coli K-12.” *Biochem Int.* 14 (1987): 227-234. (accessed May 5, 2010).

Maczulak, A. E., B. A. Behority, and D. L. Palmquist. “Effects of Long-Chain Fatty Acids on Growth of Rumen Bacteria.” *Applied and Environmental Microbiology* 42, no. 5 (November 1981): 856-862. http://aem.asm.org/cgi/reprint/42/5/856 (accessed April 28, 2010).

Mansilla, Maria C., Larisa E. Cybulski, Daniela Albanesi, and Diego de Mendoza. “Control of Membrane Lipid Fluidity by Molecular Thermosensors.” *Journal of Bacteriology* 186, no. 20 (October 2004): 6681-6688. doi:10.1128/JB.186.20.6681-6688.2004 (accessed April 28, 2010).

Merritt, Megan E., and Janet R. Donaldson. “Effect of bile salts on the DNA and membrane integrity of enteric bacteria.” *Journal of Medical Microbiology* 58 (2009): 1533-1541. doi:10.1099/jmm.0.014092-0 (accessed April 28, 2010).

Metz, James G., Paul Roessler, Daniel Facciotti, Charlene Levering, Franziska Dittrich, Michael Lassner, Ray Valentine, Kathryn Lardizabal, Frederic Domergue, Akiko Yamada, and Kazunaga Yazawa. “Production of Polyunsaturated Fatty Acids by Polyketide Synthases in Both Prokaryotes and Eukaryotes.” *Science* 293 (July 2001): 290-293. http://www.sciencemag.org (accessed April 28, 2010).

Morotomi, Masami, Yasuo Kawai, and Masahiko Mutai. “Intestinal Microflora in Rats: Isolation and Characterization of Strictly Anaerobic Bacteria Requiring Long-Chain Fatty Acids.” *Applied and Environmental Microbiology* 31, no. 4 (April 1976): 475-480. http://aem.asm.org/cgi/content/abstract/31/4/475 (accessed April 28, 2010).

Murzyn, K., T. Rog, and M. Pasenkiewicz-Gierula. “Phosphatidylethanolamine-phosphatidylglycerol bilayer as a model of the inner bacterial membrane.” *Chem. Phys. Lipids* 88, no. 2 (2005): 1091-1103. doi:10.1529/biophysj.104.048835 (accessed May 9, 2010).

Nichols, David S., Peter D. Nichols, Nicholas J. Russell, Noel W. Davies, and Tom A. McMeekin. “Polyunsaturated fatty acids in the psychrophilic bacterium *Shewanella gelidimarina* ACAM 456: molecular species analysis of major phospholipids and biosynthesis of eicosapentaenoic acid.” *Biochimica et Biophysica Acta* 1347 (1997): 164-176. doi:10.1016/S0005-2760(97)00068-4 (accessed April 27, 2010).

Nishida, Takanori, Yoshitake Orikasa, Kazuo Watanabe, and Hidetoshi Okuyama. “The cell membrane-shielding function of eicosapentaenoic acid for Escherichia coli against exogenously added hydrogen peroxide.” *FEBS Letters* 580, no. 28 (2006): 6690-6694. doi:10.1016/j.febslet.2006.11.030 (accessed April 28, 2010).

Okuyama, Hidetoshi, Yoshitake Orikasa, and Takanori Nishida. “Significance of Antioxidative Functions of Eicosapentaenoic and Docosahexaenoic Acids in Marine Microorganisms.” *Applied and Environmental Microbiology* 74, no. 3 (February 2008): 570-574. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1800774/ (accessed April 28, 2010).

Oliver, James D., and Rita R. Colwell. “Extractable Lipids of Gram-Negative Marine Bacteria: Phospholipid Composition.” *Journal of Bacteriology* 114, no. 3 (June 1973): 897-908. http://jb.asm.org/cgi/reprint/114/3/897.pdf (accessed April 28, 2010).

Palsdottir, Hildur, and Carola Hunte. “Lipids in membrane protein structures.” *Biochimica et Biophysica Acta* 1666 (2004): 2-18. doi:10.1016/ j.bbamem.2004.06.012 (accessed April 28, 2010).

Prouty, A. M., I. E. Brodsky, S. Falkow, and J. S. Gunn. “Bile-salt-mediated induction of antimicrobial and bile resistance in *Salmonella typhimurium*.” *Microbiology*, no. 150 (2004): 775-783. doi:10.1099/mic.0.26769-0 (accessed April 8, 2010).

Provenzano, D., D. A. Schuhmacher, J. L. Barker, and K. E. Klose. “The virulence regulatory protein ToxR mediates enhanced bile resistance in *Vibrio cholerae* and other pathogenic Vibrio species.” *Infectious Immunology* 69 (2000): 1491-1497. doi:0019-9567/00 (accessed May 9, 2010).

Reidl, Joachim, and Karl E. Klose. “*Vibrio cholerae* and cholera: out of the water and into the host.” *FEMS Microbiology Reviews* 26 (April 2002): 125-139. http://www.fems-microbiology.org (accessed April 7, 2010).

Schuhmacher, D. A., and K. E. Klose. “Environmental signals modulate ToxT-dependent virulence factor expression in *Vibrio cholerae*.” *Journal of Bacteriology* 181 (1999): 1508-1514. doi:0021-9193/99 (accessed May 9, 2010).

Senderovich, Yigal, Ido Izhaki, and Malka Halpern. “Fish as Reservoirs and Vectors of *Vibrio cholerae*.” *PLoS ONE* 5, no. 1 (January 2010): e8607. http://www.plosone.org (accessed April 7, 2010).

Slowey, J. Frank, Lela M. Jeffrey, and Donald W. Hood. “The fatty-acid content of ocean water.” *Geochimica et Cosmochimica Acta* 26 (1962): 607-616. doi:10.1016/ 0016-7037(62)90041-8 (accessed April 26, 2010).

Society for General Microbiology. “Bile sends mixed signals to E. coli.” *ScienceDaily*, April 6, 2010. hthttp://www.sciencedaily.com /releases/2010/03/ 100330210940.htm (accessed May 9, 2010).

Thanassi, D G, L W Cheng, and H Nikaido. “Active efflux of bile salts by Escherichia coli.” *Journal of Bacteriology* 179, no. 8 (1997): 2512-2518. doi:0021-9193/97 (accessed May 9, 2010).

Wakeham, Stuart G., John I. Hedges, Cindy Lee, Michael I. Peterson, and Peter J. Hernes. “Compositions and transport of lipid biomarkers throughg the water column and surficial sediments of the equatorial Pacific Ocean.” *Deep-Sea Research II* 44, no. 9-10 (1997): 2131-2162. doi:0967-0645/98 (accessed April 26, 2010).

Wier, Andrew M., Spencer V. Nyholm, Mark J. Mandel, R. Prisca Massengo-Tiasse, Amy L. Schaefer, Irina Koroleva, Sandra Splinter-BonDurant, Bartley Brown, Liliana Manaella, Einat Snir, and Hakeem Almabrazi. "Transcriptional patterns in both host and bacterium underlie a daily rhythm of anatomical and metabolic change in a beneficial symbiosis." *PNAS* 107, no. 5 (February 2010): 2259-2264. http://www.pnas.org/cgi/doi/10.1073/pnas.0909712107 (accessed April 26, 2010).

Willey, Joanne M., Linda M. Sherwood, and Christopher J. Woolverton. *Microbiology* . 7th ed., 986. New York: McGraw-Hill, 2008.

Wydro, Pawel, and Karolina Witkowska. "The interactions between phosphatidylglycerol and phosphatidylethanolamines in model bacterial membranes The effect of the acyl chain length and saturation." *Colloids and Surfaces B: Biointerfaces* 72 (2009): 32-39. http://www.elsevier.com/locate/colsurfb (accessed April 17, 2010).

Zhang, Yong-Mei, and Charles O. Rock. "Acyltransferases in bacterial glycerophospholipid synthesis." *Journal of Lipid Research* 49 (2008): 1867-1874. doi:10.1194/jlr.R800005-JLR200 (accessed April 26, 2010).

Zhang, Yong-Mei, and Charles O. Rock. "Membrane lipid homeostasis in bacteria." *Nature Reviews Microbiology* 6 (March 2008): 222-233. doi:10.1038/ nrmicro1839 (accessed April 26, 2010).

Zhao, W, T. Rog, A. A. Gurtovenko, A. I. Vattulainen, and M. Karttunen. "Role of phosphatidylglycerols in the stability of bacterial membranes." *Biochimie* 90 (2008): 930-938. doi:10.1016/j.biochi.2008.02.025 (accessed May 9, 2010).

Zheng, Chang Ji, Jung-Sung Yoo, Tae-Gyu Lee, Hee-Young Cho, Young-Ho Kim, and Won-Gon Kim. "Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids." *FEBS Letters* 579 (2005): 5157-5162. doi:10.1016/ j.febslet.2005.08.028 (accessed April 28, 2010).

Fatty Acid	Solvent	Stock Concentration	Final Concentration
Palmitic Acid (16:0)	Chloroform	0.5 M	0.5 mM
Heptadecanoic acid (17:0)	Chloroform	0.18 M	0.3 mM
Stearic Acid (18:0)	Chloroform	0.35 M	0.5 mM
Oleic acid (18:1)	Ethanol with 42 mg of Brij 59	0.0003 M	0.5 mM
Linoleic acid (18:2)	1% TWEEN in water with .1 M sodium phosphate buffer	0.03 M	0.5 mM
Arachidonic acid (20:4)	Methanol	0.15 M	0.5 mM
Dihomo-γ-linolnic acid (20:3)	Ethanol	0.326 M	0.5 mM
α-Linolenic acid (18:3n-3)	Ethanol	0.9 M	0.5 mM
γ-Linolenic acid (18:3n-6)	Ethanol	0.36 M	0.5 mM
Eicosapentaenoic acid (20:5)	Methanol	0.165 M	0.5 mM
Docosahexaenoic acid (22:6)	Ethanol	0.3 M	0.5 mM

Table 1. Fatty acids used in this study.

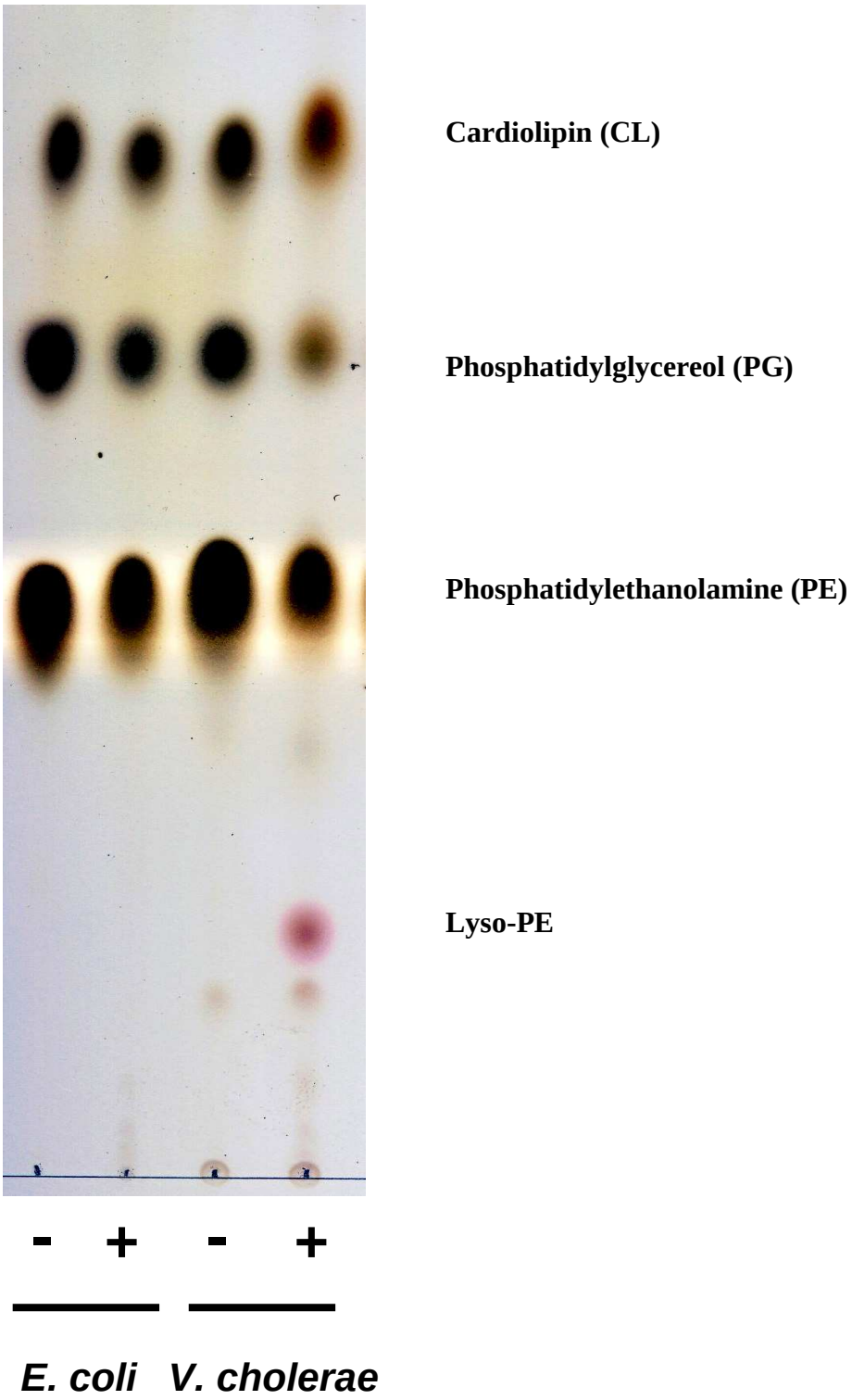


Figure 1. TLC of isolated phospholipids from *E. coli* and *V. cholerae* grown with and without 0.4% bile. Growth of *V. cholerae* in bile causes several noticeable changes in the phospholipid profile, including an upward shift in cardiolipin, a decrease in phosphatidylglycerol and phosphatidylehtanolamine and the appearance of lyso-phosphatidylethanolamine.

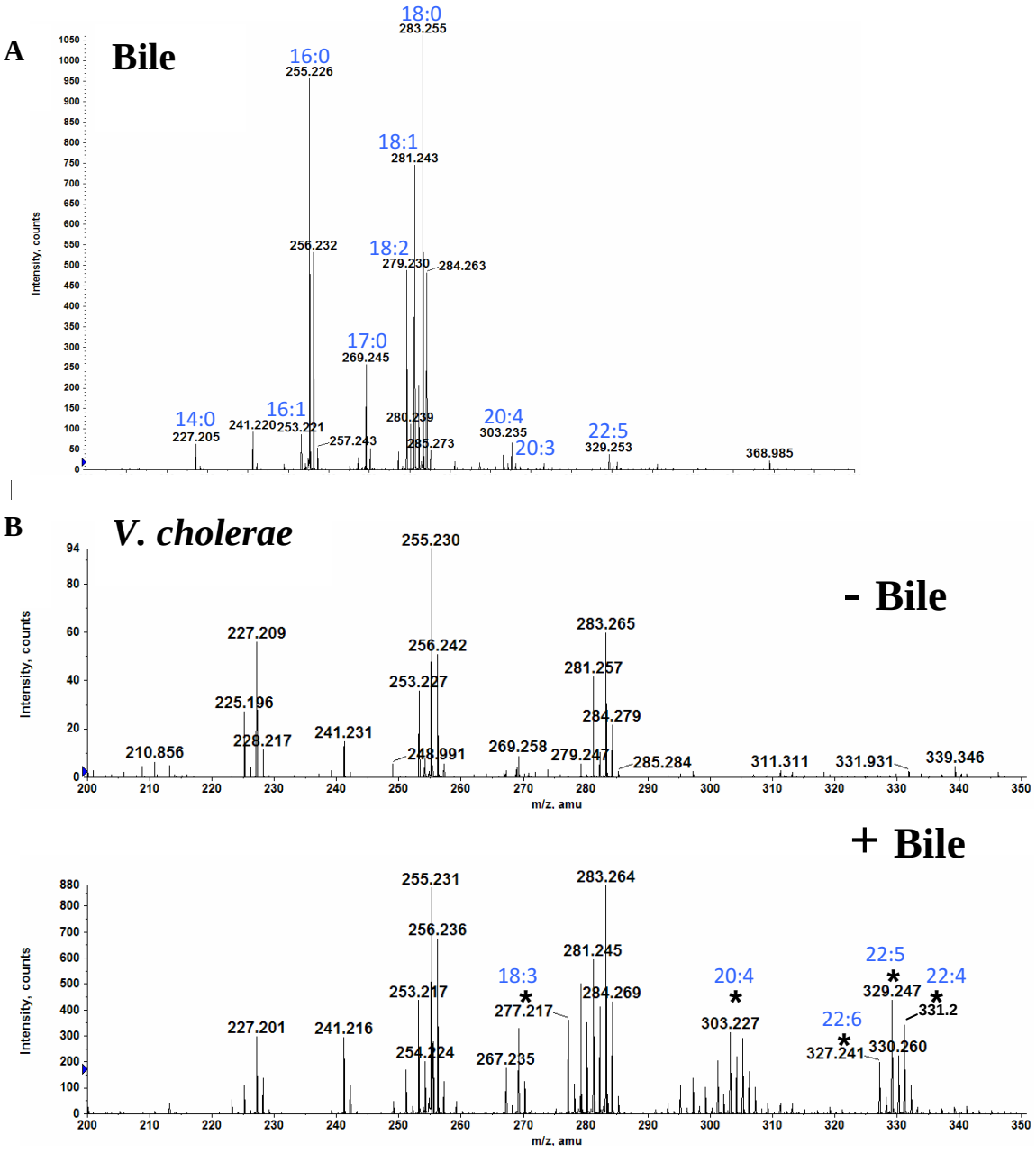


Figure 2. Mass Spectrometry of crude bile and *V. cholerae* grown with and without bile. Bile contained the fatty acids14:0, 16:1, 16:0, 17:0, 18:0, 18:1, 18:2, 20:3, 20:4, and 22:5. *V. cholerae* grown in bile contained several fatty acids not present during growth without bile. The new fatty acids include 18:3, 20:4, 22:4, 22:5, 22:6 present in addition to fatty acids in bile.

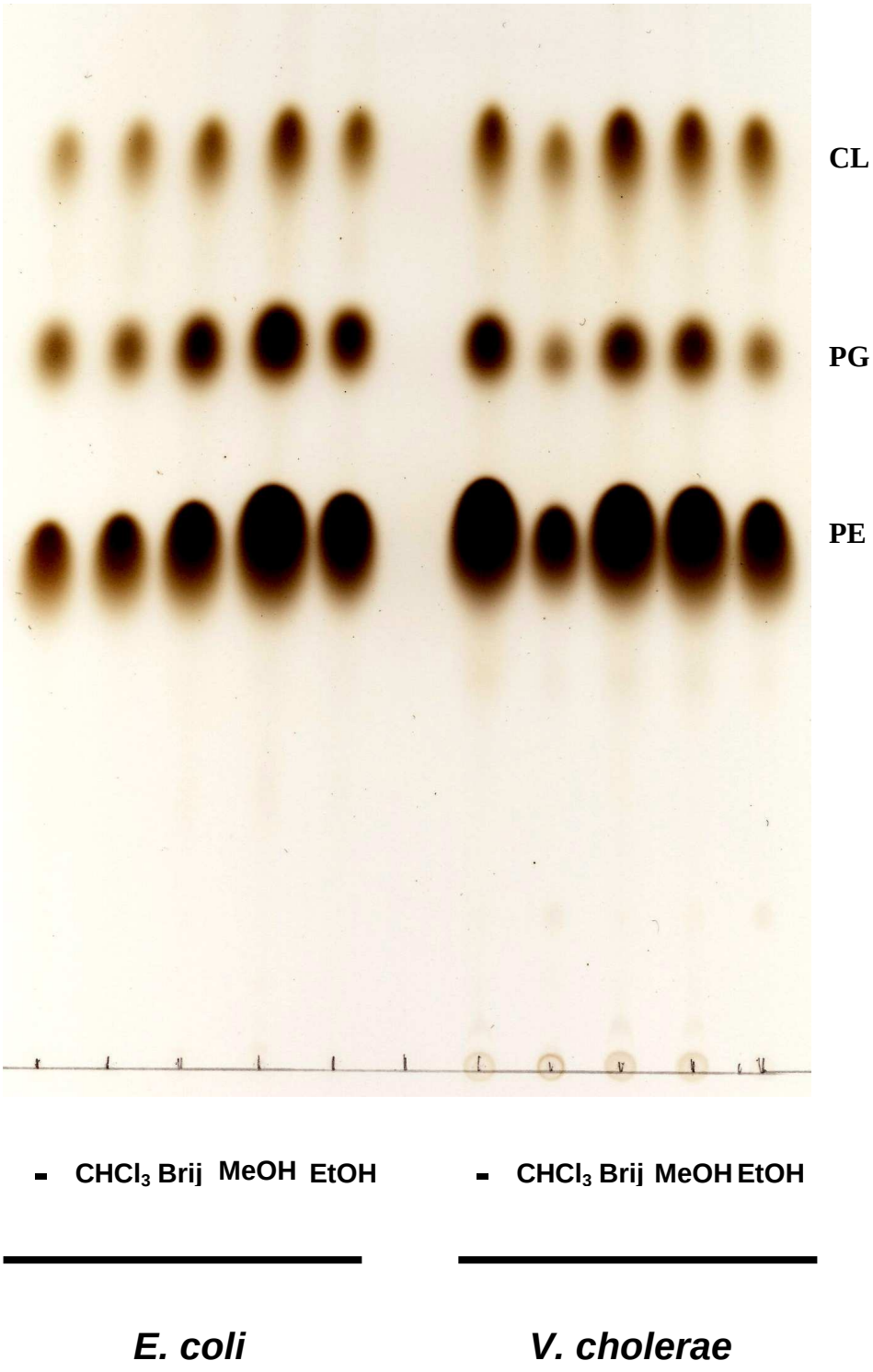
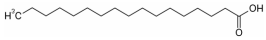


Figure 3. The solubilizing agents did not affect the phospholipid profiles of *E. coli* and *V. cholerae*. Growth of *E. coli* and *V. cholerae* in the presence of various solvents and detergents used for solubilization of individual fatty acids did not cause substantial alteration of the phospholipid profiles. Chloroform ($CHCl_3$); Brij (Brij 58); Methanol (MeOH); Ethanol (EtOH).

Palmitic acid (16:0)

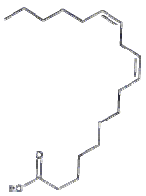
Heptadecanoic acid (17:0)



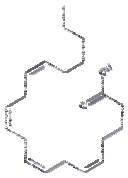
Stearic acid (18:0)

Oleic acid (18:1)

Linoleic acid (18:2)

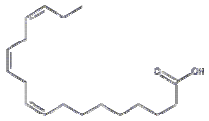


Arachidonic acid (20:4)

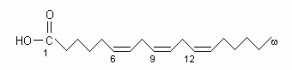


Dihomo-gamma-linolenic acid (20:3)

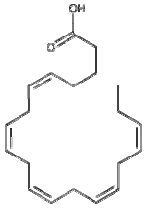
Alpha-linolenic acid (18:3n-3)



Gamma-linolenic acid (18:3n-6)



Eicosapentaenoic acid (20:5)



Docosahexaenoic acid (22:6)

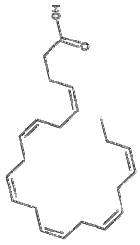


Figure 4. Structures of relevant fatty acids

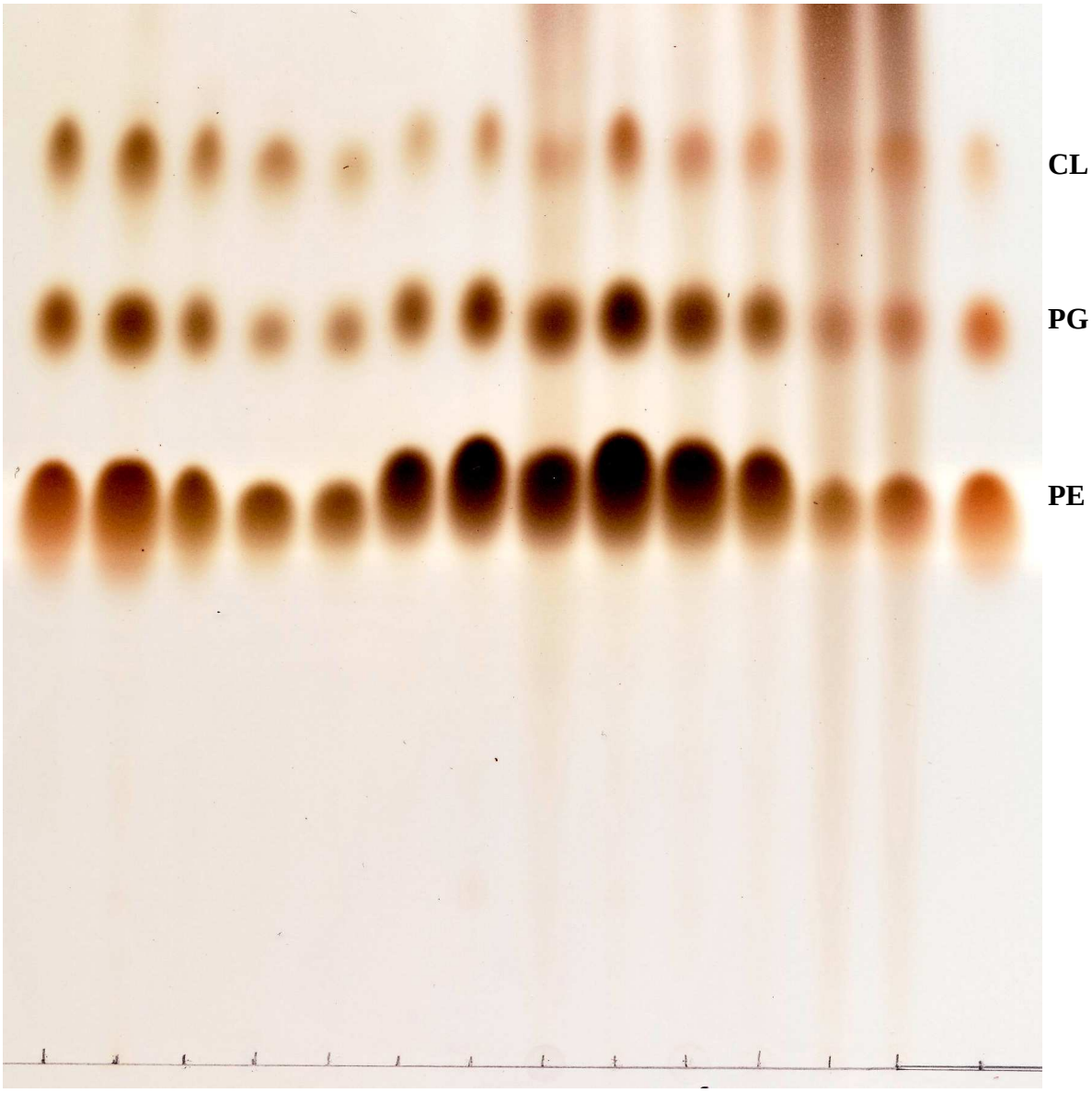


Figure 5. *E. coli* grown with 0.4% bile and fatty acids within bile. The phospholipids of *E. coli* do not undergo change. Fatty acids: palmitic acid (16:0); heptadecanoic acid (17:0); stearic acid (18:0); oleic acid (18:1); linoleic acid (18:2); α-linolenic acid (18:3:n-3); γ-linolenic acid (18:3:n-6); arachidonic acid (20:4); dihomo- γ -linolenic acid (20:3); eicosapentaenoic acid (20:5); docosahexaenoic acid (22:6).

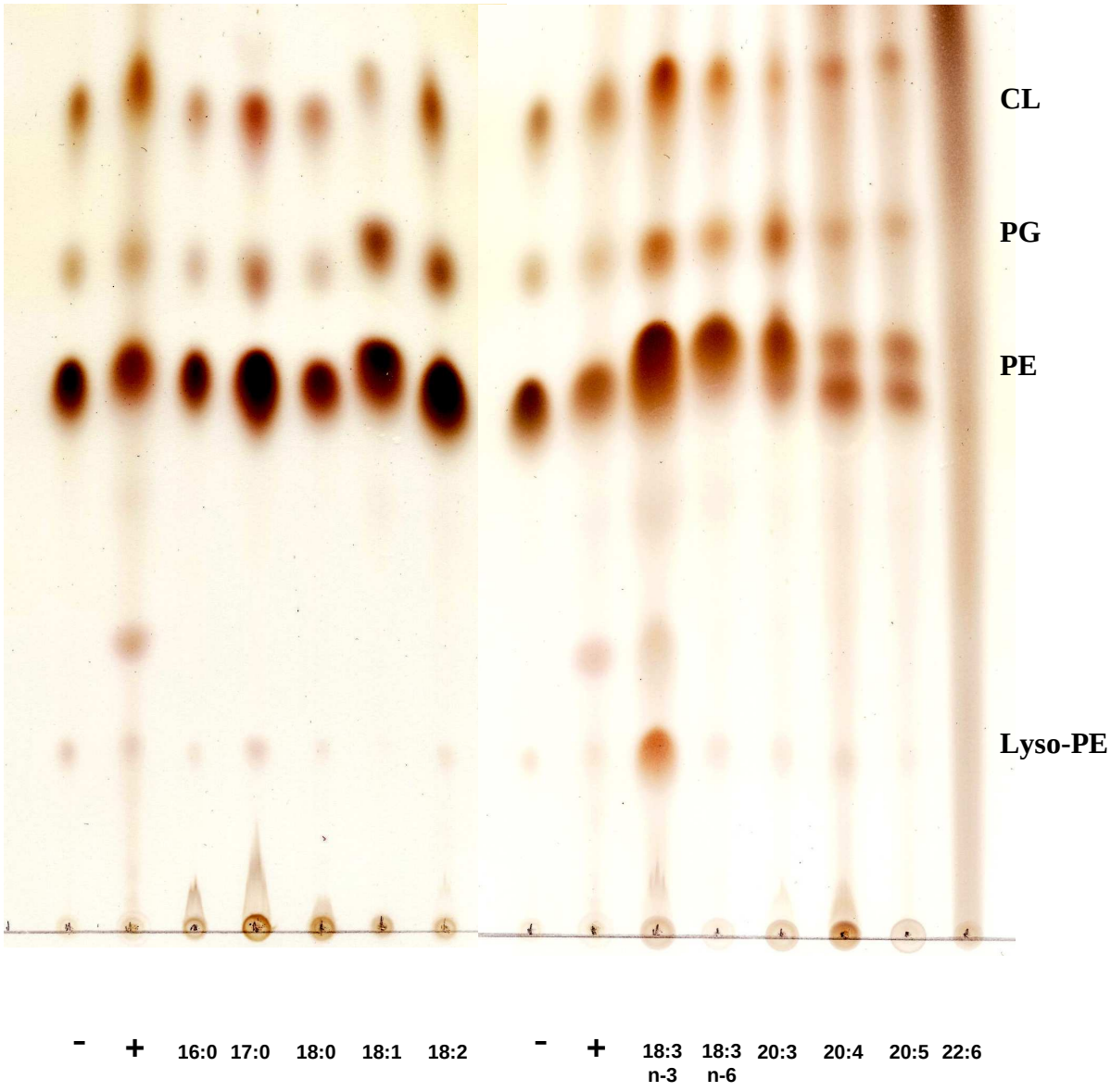


Figure 6. *V. cholerae* grown with 0.4% bile and fatty acids within bile. The *V. cholerae* phospholipid profile changes according to the chain length and saturation of each exogenous fatty acid. Fatty acids: palmitic acid (16:0); heptadecanoic acid (17:0); stearic acid (18:0); oleic acid (18:1); linoleic acid (18:2); α-linolenic acid (18:3:n-3); γ-linolenic acid (18:3:n-6); arachidonic acid (20:4); dihomo- γ -linolenic acid (20:3); eicosapentaenoic acid (20:5); docosahexaenoic acid (22:6).

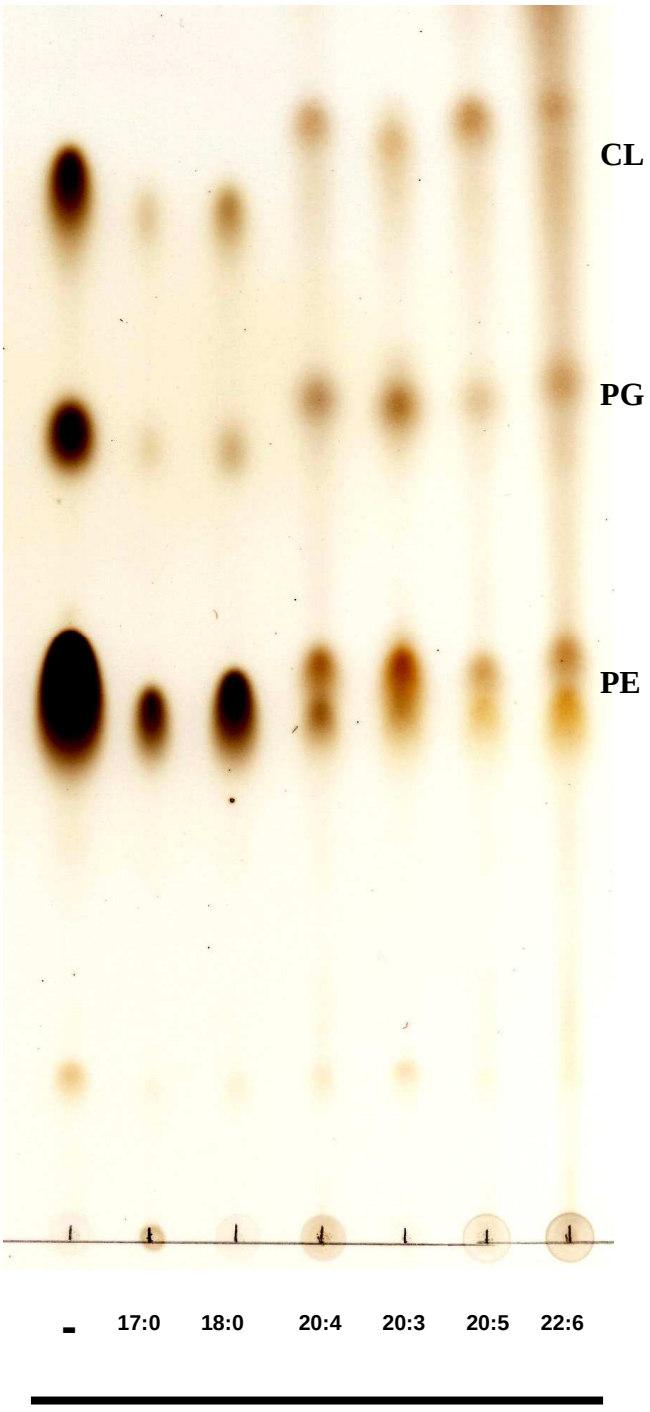


Figure 7. *V. cholerae* grown with select fatty acids within bile. *V. cholerae* phospholipids shift upward as the exogenous fatty acid increases in chain length and/or saturation. In some cases, two species of a single phospholipid can be observed. These show the production of a phospholipid species from both endogenous and exogenous fatty acid supplies. Fatty acids: heptadecanoic acid (17:0); stearic acid (18:0); arachidonic acid (20:4); eicosapentaenoic acid (20:5); docosahexaenoic acid (22:6).