Impact of Aerobic and Anaerobic Conditions on the Lipid Composition of *E. coli*

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August 2016

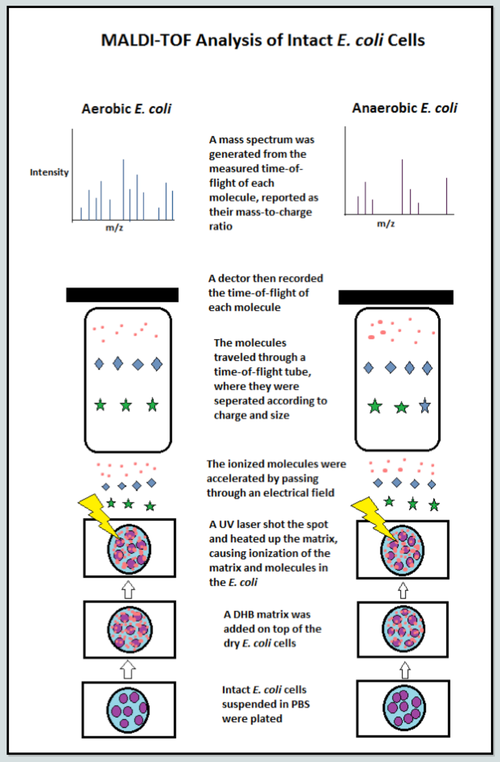
Montana IDeA Network of Biomedical Research Excellence (INBRE)

INTRODUCTION

Prokaryotes and eukaryotes are both able to produce ATP in aerobic and anaerobic conditions; however, ATP production is usually a lot more effective and efficient in aerobic conditions. Therefore, it is undesirable for cells to produce ATP anaerobically if it isn't necessary. It is understood how cells produce ATP, but the machinery associated with how ATP production is controlled and how these pathways are dynamically regulated isn't fully understood. The determination of the lipid content of *Escherichia coli (E. coli)* in aerobic and anaerobic conditions was used as an initial step to determining how ATP production is dynamically regulated.

Lipids allow an insight into what is going on inside cells when they are in environments of varying oxygen levels. They play an important role in energy balance in the body. Lipids have also been used as biomarkers for oxidative stress in cells. Therefore, investigating the lipid content of cells in aerobic and anaerobic conditions can help to determine how ATP production is dynamically regulated.

Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) is a mass spectrometer that allows for the analysis of molecules in a wide mass present in a sample. In particular it allows for the identification of intact lipids from intact cells.



METHODS

- *E. coli* was grown in total aerobic and total anaerobic conditions.
- Nitrogen gas was bubbled through the LB media and air space to create an anaerobic environment.
- A needle was used to extract the anaerobic *E. coli* from an airtight capped bottle.
- The *E. coli* was incubated at 37°C.
- A standard growth curve was created by collecting and measuring the optical density (OD) of the *E. coli* every 2 hours.
- Cell pellets were collected at the log and stationary phase.
- The lipid content of the *E. coli* cell pellets was determined using MALDI-TOF.
- The cell pellets were re-suspended in phosphate-buffered saline (PBS), because it worked better than ammonium acetate.
- 1 µL of the cell pellet slurries were spotted on the MALDI plate.
- 1 µL of DHB matrix was applied on top of the dry spots.

RESULTS

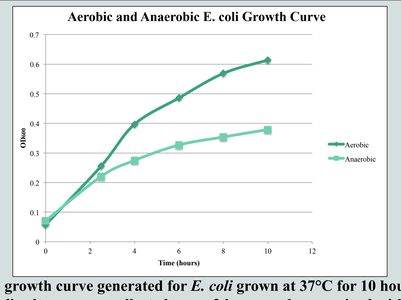


Figure 2. The growth curve generated for *E. coli* grown at 37°C for 10 hours. 500 µL of the *E. coli* cultures were collected every 2 hours and were mixed with 500 µL of LB media in a cuvette to measure the OD at a wavelength of 600 nm.

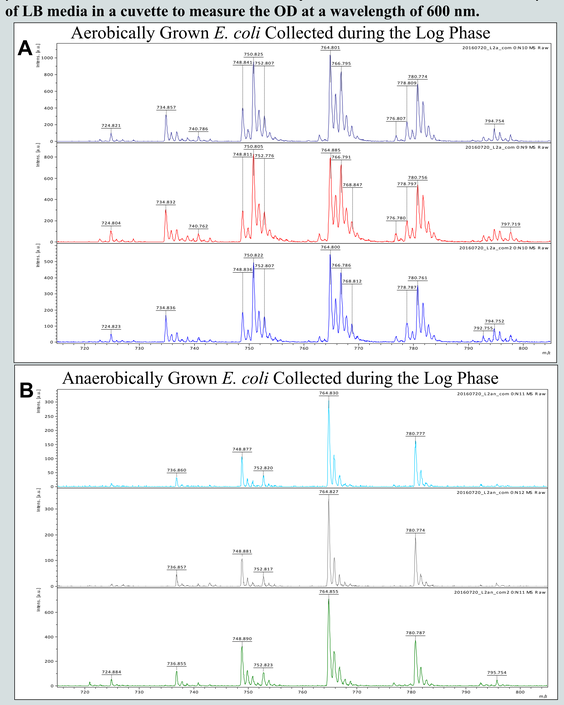


Figure 3. The spectra in figure 3 were generated by running aerobically and anaerobically grown *E. coli* collected during the log phase on the MALDI-TOF. These figures illustrate the reproducibility of the generated spectra.

DISCUSSION

When the *E. coli* cells were run on the MALDI-TOF, phospholipid phophatidylethanolamines (PE) and phosphatidylglycerols (PG) were identified. PE lipids are found in the lipid bilayer of cells and help maintain the curvature of the lipid bilayer and help stabilize membrane proteins. PE lipids also assist in the folding and movement of proteins (1). PG lipids are synthesized in the mitochondria and are the precursor for cardiolipin production, which is necessary for function of enzymes associated with oxidative phosphorylation (2).

The difference in the PE and PG lipids identified are the number of carbons and degree of unsaturation of the fatty acid chains (3).

The analysis of the lipids present in the membranes of the *E. coli* in aerobic and anaerobic conditions showed that there was more variation in the membrane lipids present in aerobic conditions. For example, 11 m/z peaks were identified as membrane lipids in the aerobic *E. coli*, but only only 3 were identified in the anaerobic. Figure 4 illustrate that less peaks were generated by the MALDI-TOF in anaerobic conditions. The most noticeable difference between the mass spectra for the aerobic and anaerobic *E. coli* was that the aerobic *E.coli* had more heterogeneity of each membrane lipid. This means that the aerobic *E. coli* had more unsaturated lipid species for each saturated lipid than the anaerobic *E. coli*. This could signify that *E. coli* produce a wider spectrum of membrane lipids when they are producing ATP aerobically.

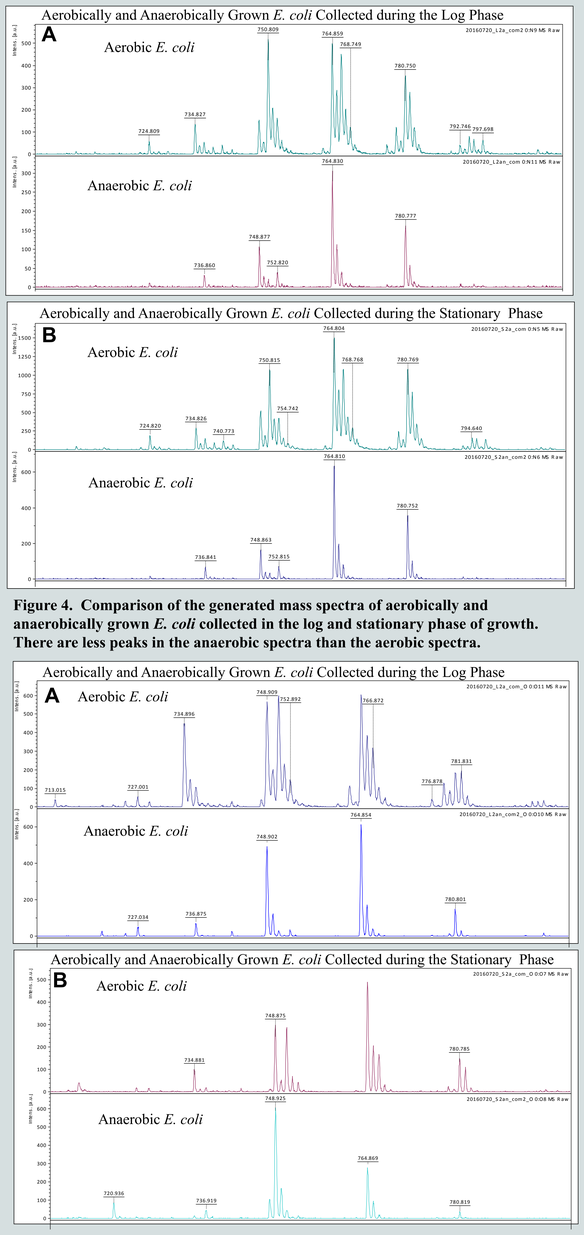


Figure 4. Comparison of the generated mass spectra of aerobically and anaerobically grown *E. coli* collected in the log and stationary phase of growth. There are less peaks in the anaerobic spectra than the aerobic spectra.

Figure 5. The generated mass spectra of *E. coli* cell pellets that were suspended in ammonium acetate instead of PBS.

It was also determined that re-suspending the *E. coli* cell pellets in PBS instead of ammonium acetate produced better mass spectra for looking at membrane lipids. The mass spectra generated from *E. coli* suspended in ammonium acetate and PBS both had a greater number of peaks identified as lipids in aerobic conditions. However, the mass spectra of *E. coli* in PBS allowed for a greater number of lipid identifications.

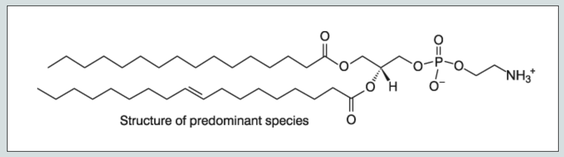


Figure 6. This is an illustration of the structure of PE (33:1), one of the membrane lipids present in *E. coli*.

	m/z	Lipid
Log Phase Aerobic	602.8	PE (24:0) [M+Na]+
	734.5	PE (32:1) [M+2Na-H]+
	736.8	PE (32:0) [M+2Na-H]+
	740.8	PE (34:2) [M+Na]+
	748.8	PE (33:1) [M+2Na-H]+
	765.8	PE (36:0) [M+NH_4]+
	766.8	PE (36:2)
	768.8	PE (36:1)
	776.8	PE (35:1) [M+2Na-H]+
	778.8	PG (36.0) [M•]
	779.8	PG (36:0) [M+H]2+
	m/z	**Lipid**
Log Phase Anaerobic	736.9	PE (32:0) [M+2Na-H]+
	748.8	PE (33:1) [M+2Na-H]+
	766.7	PE (36:2)

	m/z	Lipid
Stationary Phase Aerobic	602.8	PE (24:0) [M+Na]+
	734.8	PE (32:1) [M+2Na-H]+
	736.8	PE (32:0) [M+2Na-H]+
	740.7	PE (34:2) [M+Na]+
	748.8	PE (33:1) [M+2Na-H]+
	754.7	PE (35:1) [M+Na]+
	765.8	PE (36:0) [M+NH_4]+
	766.8	PE (36:2)
	768.8	PE (36:1)
	778.8	PG (36.0) [M•]
	779.8	PG (36:0) [M+H]2+
	m/z	**Lipid**
Stationary Phase Anaerobic	602.8	PE (24:0) [M+Na]+
	736.9	PE (32:0) [M+2Na-H]+
	748.8	PE (33:1) [M+2Na-H]+

Figure 7. These tables show the identification of each generated m/z peak with its correlated lipid (3,4,5).

CONCLUSIONS

- Intact diacylphospholipids can be analyzed directly from intact cells.
- There is little difference in the lipid content of the membranes' of *E. coli* in the log and stationary phases of growth.
- *E. coli* grown under aerobic conditions contain a greater heterogeneity of membrane lipids than the anaerobic *E. coli*.
- *E. coli* grown under aerobic conditions showed more degrees of unsaturation in their identified membrane lipids.
- *E. coli* produces a more varied membrane lipid bilayer in aerobic conditions.
- The greater heterogeneity in the aerobically grown *E. coli* could be a result of more access to electron donating molecules to drive elongation and double bond formation.
- The metabolism and growth of fatty acids in aerobically grown *E. coli* is regulated by the FadR, but anaerobically grown *E. coli* is not strongly regulated by the FadR protein. This could be an explanation for the difference in the membrane lipid composition.

FUTURE WORK

- Determine the significance of the difference in the lipid composition and variation of the membrane lipids of *E. coli* grown aerobically and anaerobically.
- Investigate if the membrane lipid content is affected by the transition of *E. coli* between aerobic and anaerobic conditions.
- Determine the lipid content of human skeletal muscle tissue in aerobic and anaerobic conditions using MALDI-IMG.

ACKNOWLEDGMENTS/ REFERENCES

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Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103474. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The Mass Spectrometry Facility receives support from the Murdock Charitable Trust.