Mycobacterium tuberculosis Protein PPE51 Interactions with Glucose and Glycerol

Megan R. Murto, Shelby J. Dechow, Robert Abramovitch
Department of Microbiology and Molecular Genetics, Michigan State University

Introduction

Using *E. coli* as a model

- *Mycobacterium tuberculosis* (Mtb), the pathogen that causes tuberculosis, kills over 1.5 million people every year.
- Tuberculosis has a doubling rate of 24 hours under the best of conditions, making this a very slow growing microbe.
- *Escherichia coli* is often used as a model in place of Mtb because of it's fast growing times, with a doubling rate of 20 minutes, as well as the lower pathogenicity, making this a much safer microbe to work with in a BSL-2 laboratory.

Why focus on protein PPE51, glucose, and glycerol?

- In a previous experiment, it was found that wild-type PPE51 is associated with the uptake of disaccharides in Mtb. [1]
- When the wild-type PPE51 was placed in minimal media supplemented with glucose, there was no significant difference in growth. On the other hand, glycerol was found to increase the growth kinetics in several mutants. [1]
- It's proposed that both glucose and glycerol are associated with protein stability at higher temperatures.

Abstract

Escherichia coli is commonly used in place of *Mycobacterium tuberculosis* (Mtb) due to its lower pathogenicity and faster doubling rate, allowing for more convenient BSL-2 laboratory access and a shorter growth window. Previously, it was found that Mtb uses the PPE51 protein to take up disaccharides into the cell. Upon further experimentation, it was found that when glucose was supplemented into minimal media, the growth kinetics were not changed. On the other hand, glycerol was found to increase the growth of the PPE51 CRISPER-strong mutants. From this, it was hypothesized that different carbon sources, specifically glucose and glycerol, would stabilize the PPE51 protein. Wild type (WT) *E. coli* expressed the Mtb protein PPE51 from the T7-inducible pET plasmid and a thermostability assay was used to examine the protein degradation at different temperature levels in 5℃ increments, ranging from 35℃ to 60℃. This experiment concluded that glycerol is used in order to stabilize the PPE51 protein at higher temperatures. Glucose is theorized to not stabilize the protein at higher temperatures, however the experiment should be repeated due to difficulties with the SDS-PAGE gel.

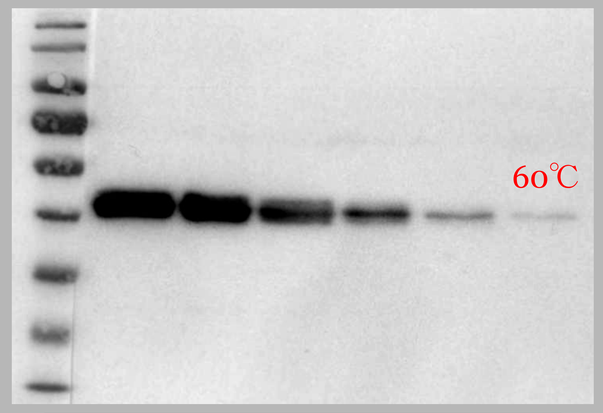

Figure 1: PPE51 protein degradation with 10mM glycerol solution added

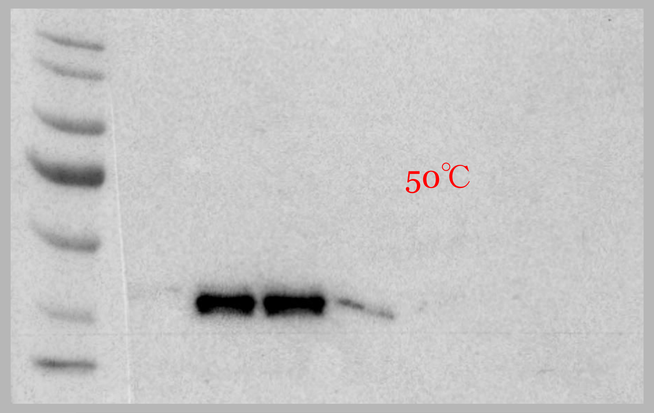

Figure 2: PPE51 protein degradation with 10mM glucose solution added

Results

Glycerol (Figure 1)

- Starting from left to right, the wells are as follows: Page Ladder, room temperature, 35℃, 40℃, 45℃, 50℃, 55℃, and 60℃.
- In the WT with no glycerol or glucose added, protein degradation was most apparent at 55℃.
- In the WT with 10 mM glycerol solution added, protein degradation is most apparent at 60℃ (well 7).

Glucose (Figure 2)

- Starting from left to right, the wells are as follows: Page Ladder, room temperature, 35℃, 40℃, 45℃, 50℃, 55℃, and 60℃.
- In the WT with no glycerol or glucose added, protein degradation was most apparent at 55℃.
- In the WT with 10 mM glucose solution added, protein degradation is most apparent at 50℃, where a slight band can be seen (well 5).
- This could be due to the fact that the SDS-PAGE gel tore when transferring to the Western Cassette.

1. Korycka-Machała M, Pawełczyk J, Borówka P, et al. PPE51 Is Involved in the Uptake of Disaccharides by *Mycobacterium tuberculosis*. *Cells*. 2020;9(3):603. Published 2020 Mar 3. doi:10.3390/cells9030603

Methods

Expression, Growth Conditions, and Purification

- Mtb protein PPE51 expressed from *E. coli* BL21 (DE3) from the T7-inducible pET plasmid.
- Wild-type was induced in LB broth overnight, sonicated, and purified via batch column purification.

Thermostability and Imaging

- Wild-type PPE51 was subjected to various temperatures for a 5 minute long period. One batch was conducted without glucose while another was done in a 10 mM glucose solution or a 10 mM glycerol solution, depending on the test performed.
- Temperatures involved included room temperature as well as 5℃ increments from 35℃-60℃.
- The thermostability assay was imaged via western blot imaging.

Conclusions

Results

- Glycerol interacts with PPE51 to stabilize the protein at higher temperatures.
- Glucose is theorized not produce any stability for the protein.

Future Directions

- Glucose thermostability assay should be repeated due to gel tearing when transferring to the Western Blot transfer cassette.
- Use other forms of carbon sources and/or antibiotics in order to determine what PPE51 interacts with and uptakes.
- A thermal shift assay in order to further measure protein stability.