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Dynamic Effects on External pH and Anaerobic Growth of E. coli: Varied Carbon Sources and Hydrogenase Mutation

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Dynamic Effects on External pH and Anaerobic Growth of *Escherichia coli*: Varied Carbon Sources and Hydrogenase Mutation

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Abstract:

This study found that hydrogenase mutation does affect the growth and pH maintenance of *Escherichia coli* grown anaerobically. The *E. coli* K-12 W3110S and PMD23 cultures were grown in casein hydrolysate with a supplementary carbon source and minimal salts at pH 5.0 and 8.5. Based on previous expression data, *hypF* should not be necessary for normal growth at pH 8.5 (Hayes *et al*., 2005 manuscript in progress). However, this study found the opposite to be true. Both pH and growth differences were observed when comparing the parent and mutant strains at pH 8.5; validating the importance of hydrogenase. The effects of the mutation were also present in growth at pH 5.0, but to a lesser extent, as PMD23 cells grew to optical densities near the parent at the low pH. Overall, hydrogenase mutants had lower growth, especially at pH 8.5, and were not able to shift the external pH as much as the parent strain. Growth on different carbon sources led to mixed results, and many additional experiments will be required to ascertain the exact role of each carbon source.

Question:

How does a hydrogenase mutant differ in growth and pH maintenance compared to the wild type when grown on different carbon sources?

Introduction:

Escherichia coli is part of the normal body flora, but a pathogenic strain is often associated with outbreaks of food poisoning causing illness and death. Understanding how the bacteria survive in the human body under various conditions is essential to forming effective treatments. Enterobacteria, such as *E. coli*, are able to live in both aerobic conditions and anaerobically when air is not available, such as in the human digestive tract or aquatic environments (Souza et al., 2002). Though the 0157:H7 strain of *E. coli* poses a threat to human health, another variety known as K-12 is harmless and thus often used in scientific studies. My experiments will focus on W3110S and PMD23 *E. coli*, non-pathogenic strains derived from K-12.

Within the human digestive tract, *E. coli* cells are forced to navigate a wide array of pH conditions. First they must survive the stomach at pH 1 to 2 (fasting) or pH 2 to 7 (feeding), then pancreatic secretions at pH 10. Once in the intestine, the home of *E. coli* in the human body, pH levels of 5 to 8 must be tolerated for growth and life extension (Maurer et al., 2005). While growing in those conditions, *E. coli* are able to maintain an internal pH (7.3-7.8).

If *E. coli* is forced to grow in significantly less than optimal cytoplasmic pH conditions of higher or lower than 7.6, protective responses will be triggered. The goal of these responses is to either maintain the internal pH or prepare the cell for future exposure to extreme pH levels (Maurer et al., 2005). Gene expression shifts significantly when a stress response occurs in the cell. Previous gene expression studies by the Slonczewski lab (Maurer et al., 2005 and Hayes et al., 2005 manuscript in progress) have shown marked effects on *hypF* expression in both aerobic and anaerobic conditions. The gene was found to be induced at high pH in aerobic conditions, and induced at low pH under anaerobic conditions.

Gene *hypF* encodes a protein that is key to the maturation of hydrogenase. Hydrogenase is responsible for converting formate formed in a pathway during fermentation under anaerobic conditions (Sawers *et al*., 2004). Hydrogenase creates molecular hydrogen from the formate, which can then be expelled from the cell (Blokesch *et al*., 2004 and Casalot *et al*., 2001). My project explored the effects of hydrogenase mutant growth on multiple carbon sources (Fig. 2) under anaerobic conditions at high and low pH.

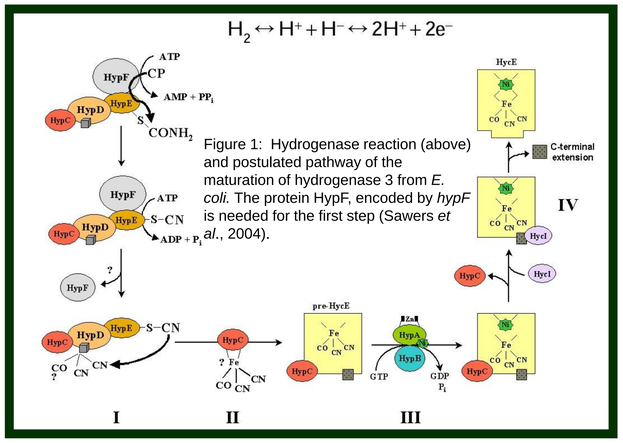


Figure 1: Hydrogenase reaction (above) and postulated pathway of the maturation of hydrogenase 3 from *E. coli*. The protein HypF, encoded by *hypF* is needed for the first step (Sawers *et al*., 2004).

Materials and Methods:

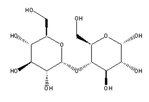
Cell growth and Analysis:

Overnight cultures of W3110S (parent) and PMD23 (hydrogenase mutant) were prepared by placing 2mL of overnight media (specially created media) into metal-capped test tubes. Overnights were allowed to rotate in incubator overnight at 37°C. Then, a 500-fold dilution transfer of overnight into three screw cap replicates filled with approximately nine mL of growth media for pH 5.0 and 8.5. Screw cap test tubes with bacteria were allowed to rotate end over end in the incubator for 24 hours (48h for sorbitol). After being read in the spectrometer the test tubes were emptied into individual plastic beakers and the pH was measured.

Figure 2: Carbon sources added to the media to test growth and pH maintenance.

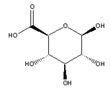
Sugars

Glucose

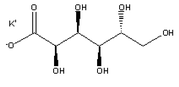


Maltose

Sugar Acids

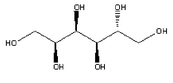


Glucuronate

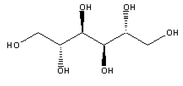


Gluconate

Sugar Alcohols



Sorbitol



Mannitol

Chemfinder.com

Results and Discussion:

Overall growth rates of the hydrogenase mutant were lower than those of the parent, remarkably so in some pH 8.5 medias. Cells grown in media with added glucose show the strongest growth and largest pH shifts. This was expected due to glucose being widely recognized as the best carbon source for metabolism in E. coli. In glucose, the pH shifted from 5.0 to 4.4 for both strains, and from 8.5 to 6.0 for W3110S. hypF mutant (PMD23) cells grown at pH 8.5 saw a shift down to pH 6.5, significantly less than the parent. Cells grown in maltose saw a pH shift to 4.55 from 5.0 for W3110S and stayed neutral in the mutant. The lack of pH movement in the mutant is most likely due to low growth. Maltose cells grown at pH 8.5 saw external pH drop to 5.2 in the parent, and 7.9 in the mutant. Again, some of this difference may be related to growth, but the pattern suggests a maltose processing deficiency in PMD23. It seems that the hydrolysis step to split maltose into two glucose molecules could be affected.

Sorbitol cultures needed 48 hours to reach growth levels comparable to those of the other carbon sources. This was observed in both the parent and mutant strain, suggesting that E. coli has a problem either transporting sorbitol into the cell or in the processing of the molecule. PMD23 grew to higher OD values in sorbitol than W3110S, the only carbon source to reverse the trend. This is a mystery, but points to hydrogenase playing a close role in sorbitol catabolism through an accessory role. The pH of sorbitol cultures increased slightly growing at pH 5.0 and pH 8.5; further adding to the uniqueness of sorbitol.

Glucuronate and gluconate cultures remained at pH 5.0 for W3110S and rose slightly in the mutant. Growth at pH 8.5 saw a shift to 7.0 in the parent and no change in the mutant. Mannitol cultures shifted to pH 4.6 and 4.5 for the parent and mutant strains grown at pH 5.0. Growth at pH 8.5 saw a downshift to 6.6 and 6.75 respectively. These results are quite different than those of sorbitol. This seems odd, as mannitol and sorbitol are stereoisomers.

The results of this study vary widely and are at times difficult to interpret; however, the importance of carbon sources and hydrogenase on external pH and growth has been shown. Further experiments are needed to fully understand the lower growth rate of PMD23 cells. One theory involves hydrogen expulsion limitations. W3110S cells grown in the various carbon sources produce varying levels of hydrogen gas (Tate, 2005 unpublished data). If cultures of PMD23 cells are found to be producing little or no gas, a link may be made to growth level. Additionally, free nickel may build up in the cell due to a breakdown in the hydrogenase maturation pathway (Fig. 1), leading to lower growth. As for the pH shift differences, only a multitude of additional experiments will find the key.

Future Steps:

- Measurement of cellular hydrogen with hydrogen electrode system to assess loss of hydrogen exportation capacity due to hydrogenase mutation.
- Repetition of experiments in micro plates using micro pH meter to eliminate possible effects of pressure buildup in anaerobic tubes.
- Measure levels of free nickel and CO/CN in mutant cells.

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Figure 3: Final pH values after growth at pH 5.0

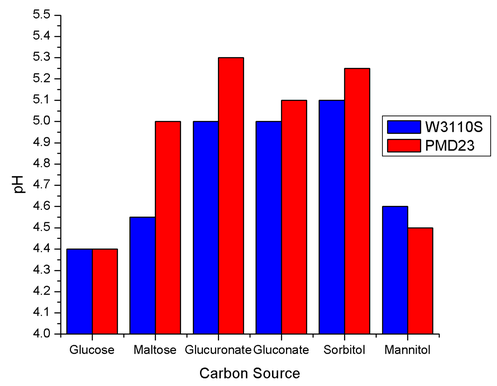
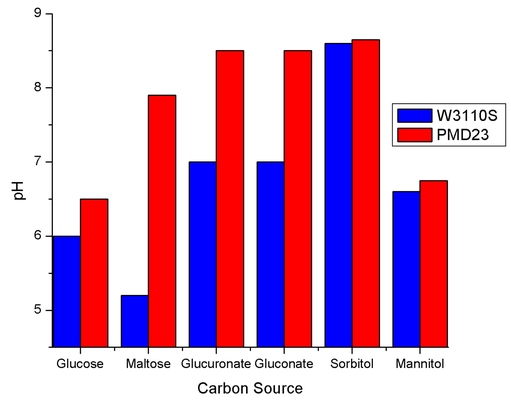


Figure 4: Final pH values after growth at pH 8.5



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