Investigation of the polyol pathway in *Entamoeba histolytica*

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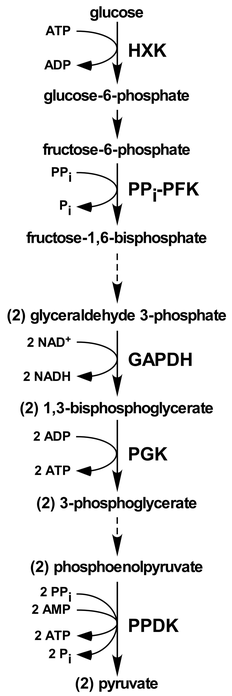
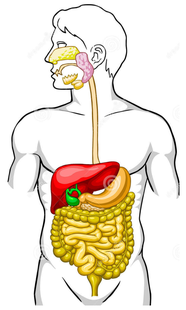
Energy Metabolism of *E. histolytica*

Entamoeba histolytica is an anaerobic parasitic amoebozoan which causes amoebic dysentery in many developing countries. However, the specific energy metabolism of this organism is still unknown. This study investigates the involvement of the polyol pathway, which consist of aldose reductase, sorbitol dehydrogenase and fructokinase as a possible solution to the energy imbalance in *E. histolytica's* modified glycolytic pathway.





E. histolytica produces both ethanol and acetate during growth on glucose. Pyruvate is first converted to acetyl-CoA by pyruvate:ferredoxin oxidoreductase (PFOR), which can then be converted to ethanol by a bifunctional aldehyde-alcohol dehydrogenase (ADHE). However, this reaction raises the concern of an energy imbalance with more NADH being consumed than produced.

Methods

- Assaying for AR consisted of 0.1M Tris pH 7.0, 500mM glucose, 1mM NADPH and 20µL cell extract. Reaction run time was 30 mins at 37 °C.
- Assaying for SD consisted of 0.1M Tris pH 7.0, 500mM glucose, 1mM NAD and 20 µL cell extract. Reaction run time was 30 mins at 37 °C.
- PCR for fructokinase consisted of 26µL molecular biology grade water, 5µL 10X PCR buffer, 5µL dNTP mix, 2µL MgSO4, 5µL 5' primer, 5µL 5' primer, 1µL *E. histolytica* genomic DNA and 1µL KID DNA polymerase.

Polyol Pathway

Aldose reductase

glucose + NADPH ↔ sorbitol + NADP

Sorbitol dehydrogenase

sorbitol + NAD^+ ↔ fructose + NADH

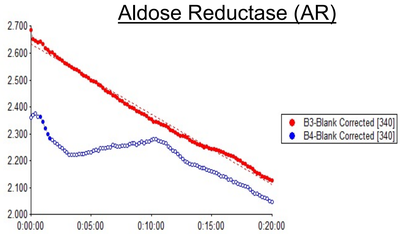
Fructokinase

fructose + ATP ↔ fructose-6-phosphate + ADP

The polyol pathway has been proposed as a possible solution to the energy imbalance in E. histolytica's modified glycolytic pathway. The genome of E. histolytica has three aldose reductase genes and a fructokinase gene, but a sorbitol dehydrogenase gene has not yet been identified. This set of reactions involves the reduction of glucose to sorbitol, which is subsequently oxidized to fructose with NADH as a byproduct. The fructose then re-enter glycolysis through fructokinase.

Aldose Reductase and Sorbitol Dehydrogenase Activity

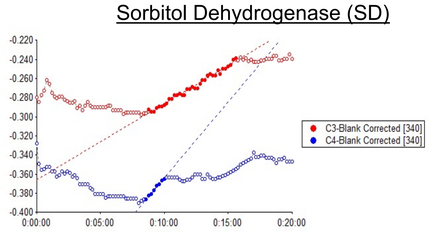
Aldose Reductase (AR)



Results: Mean V [Blank Corrected [340]] Edit

Well	Mean V [Blank Corrected [340]]	Y Intercept [Blank Corrected [340]]	R-Squared [Blank Corrected [340]]
B3	-26.015	2.634	0.991
B4	-101.898	2.445	0.992

Sorbitol Dehydrogenase (SD)



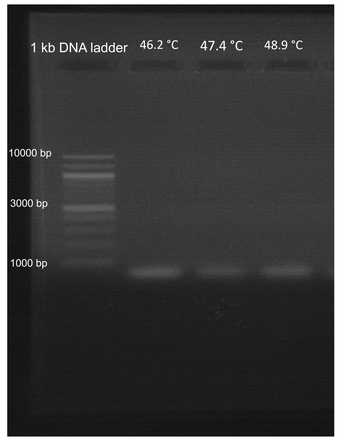
Results: Mean V [Blank Corrected [340]] Edit

Well	Mean V [Blank Corrected [340]]	Y Intercept [Blank Corrected [340]]	R-Squared [Blank Corrected [340]]
C3	7.944	-0.366	0.984
C4	15.858	-0.522	0.990

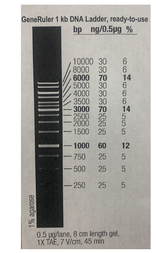
Preliminary enzymatic assaying of AR (top) and SD (bottom) activity suggest that both reactions play a role in *E. histolytica* metabolism. The AR reaction increases its NADPH consumption almost 4 times when all substates are present (blue) when compared to the absence of glucose (red). Similarly, the SD reaction increases its NADH production almost 2 times when all substates are present (blue) when compared to the absence of sorbitol (red).

Fructokinase Annealing Temperature

Fructokinase (FRK) primers showed optimal cloning temperatures at 46.2, 47.4 and 48.9 °C using the Wizard Genomic DNA Purification kit. Each sample was compared to a 1 kb DBA ladder to confirm the presence of a fructokinase gene (885 bp).



PCR Gel for FRK primers



1 kb DNA Ladder reference

Conclusions and future directions

Fructokinase primers were found to have an optimal clonal temperate at 46.2 °C. Transformation of fructokinase cells successfully produced colonies on agar plates and will be confirmed through PCR at 46.2 °C. Assaying confirmed some activity of aldose reductase and sorbitol dehydrogenase activity, however, further test and modifications must be conducted to positively conclude the polyol pathway as a solution to the NADH imbalance.

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