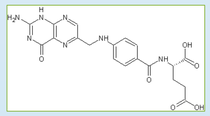
Deletion of Folate Biosynthesis Gene, *ABZ1*, Produces Transient Life-span Extension in Budding Yeast

Nausheen Qureshi and Rebecca Seipelt-Thiemann (Faculty Mentor)

Abstract

Folate, also known as vitamin B9, is essential for cellular processes in many organisms, including humans and nematodes. A prior study found that reduced folate levels led to an increase in nematode lifespan. The purpose of this study was to determine if folate biosynthesis mutations could also affect lifespan in the model organism, budding yeast. Yeast have five folate biosynthesis genes, one of which is the *ABZ1* gene. This gene encodes an enzyme that converts chorismate to 4-amino-4-deoxychorismate. Deletion of the gene produced a viable yeast, so it is not an essential gene. wildtype and *abz1* mutant yeast were grown and aged in 96 well plates. The viability of yeast in these aging plates was assayed each week for 7 weeks. Briefly, aged yeast were inoculated into fresh medium and growth was measured after 24 hours by optical density. The null hypothesis was that there is no statistically significant change in between the mutant and wildtype populations using T-tests and they are both behaving the same way. The null hypothesis was not rejected for weeks 1-4 and week 7, indicating both yeasts were behaving similarly. However, the null hypothesis was rejected for week 5, showing the mutant and wildtype populations behaved significantly different. Since the mutant change in optical density was greater than the wildtype change in optical density, the mutation caused the mutant yeast to live longer. Week 6 data was lost due to a technical error in plate reading and so its data is not included, but it would have been of considerable interest. Future studies should include repeated trials with single mutants to ensure the results are consistent and trials with double and triple mutants to determine if the effect is cumulative.

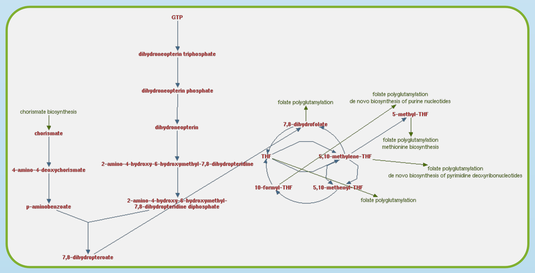


Natural Health Products Ingredients Database

Folic acid in diet, 2015

Guiding Questions

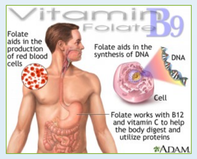
1) Is the longevity of yeast affected by the mutation in folate biosynthesis?
2) Is more folate always better?
3) How is this study applicable to species other than yeast (ex. Humans)?



Cherry, 2007

Folate and Aging

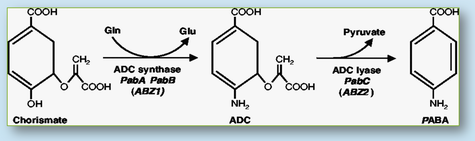
Folate is a vitamin supplement used in numerous foods that is used as a co-factor in many important cellular processes. It is also utilized to reduce the incidence of neural tube defects in humans. Folate came to the attention of aging researchers when a genetic screen in the nematode, *Caenorhabditis elegans*, was undertaken to identify genes affecting lifespan. Long-lived nematodes were identified, but it became clear that the mutation was not in the nematode, but its food source (Virk, 2012). Nematodes fed a strain of *Escherichia coli* deficient in folate biosynthesis had an increased lifespan. In addition, compounds that either inhibited folate biosynthesis or reduced its availability also increased lifespan. This research has suggested that optimum levels of folate increases longevity, while excess levels may decrease lifespan. The goal of this project was to determine the effect of one of three viable mutations in the folate biosynthesis pathway on longevity in the model organism, budding yeast.



Folic acid in diet, 2015

ABZ1 and Folate Biosynthesis

- Five genes encode enzymes in the folate biosynthesis pathway in budding yeast (Cherry, 2012)
- Of these five genes, *ABZ1*, *ABZ2*, and *MIS1* mutants are viable in yeast
- *ABZ1* encodes an enzyme that converts chorismate to 4-amino-4-deoxychorismate
- Mutating gene could cause either an increase or decrease in longevity



Botet, 2007

Hypotheses

- **Null Hypothesis:** There is no statistically significant change in between the mutant and wildtype populations and they are both behaving the same way.
- **Alternative Hypothesis:** There is a statistically significant change in between the mutant and wildtype populations and they are therefore behaving differently.

Aging Methods

- **Step 1:** Cultured mutant and wildtype yeast on solid medium for 2 days
- **Step 2:** Inoculated a single colony of mutant and wildtype yeast into liquid medium and grew for 3 days
- **Step 3:** Transferred yeast to aging plate and grew for one week (4 inoculations per strain)

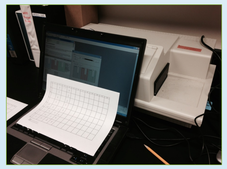
Adapted from Powers, 2006

Lifespan Assay Methods

Each week mutant and wild type yeast were inoculated into fresh medium in 96 well plates (3replicates per aging well)

A_{600} was taken at 0 hours with medium only used as a blank

A_{600} was taken at 24 hours with medium only used as a blank



Adapted from Powers, 2006

Data Analysis Methods

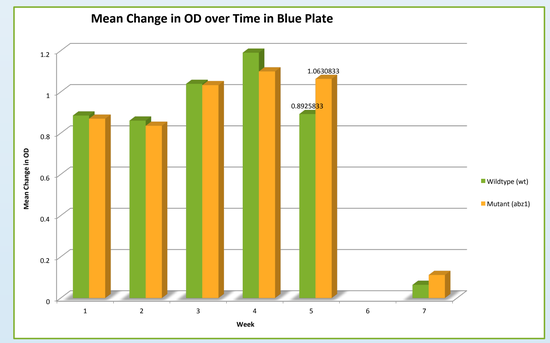
- **Step 1:** Growth data was collected each week for 7 weeks using a plate reader and exported into an excel spreadsheet
- **Step 2:** Excel was used to calculate mean, standard deviation, and t-test for each week's data
- **Step 3:** The calculated t-test value was evaluated for significance

Sample	Wells	Sample#	Pre	Post	Change		mut	(x-mean)	sq	wt	(x-mean)	sq
abz1	A4	1	0.248	1.001	0.753		0.753	-0.11692	0.01367	0.912	0.02675	0.000716
	B4		0	0.461	0.461		0.461	-0.40892	0.167213	0.89	0.00475	2.26E-05
	C4		0.022	1.025	1.003		1.003	0.133083	0.017711	0.987	0.10175	0.010353
	D4		0.014	1.032	1.018		1.018	0.148083	0.021929	0.998	0.11275	0.012713
	E3		0.017	0.933	0.916		0.916	0.046083	0.002124	0.87	-0.01525	0.000233
	E4		0.015	0.9	0.885		0.885	0.015083	0.000228	0.809	-0.07625	0.005814
	F3		0.017	0.865	0.848		0.848	-0.02192	0.00048	0.935	0.04975	0.002475
	F4		0.039	0.849	0.81		0.81	-0.05992	0.00359	0.782	-0.10325	0.010661
	G3		0.017	0.955	0.938		0.938	0.068083	0.004635	0.863	-0.02225	0.000495
	G4		0.056	0.877	0.821		0.821	-0.04892	0.002393	0.795	-0.09025	0.008145
	H3		0.037	1.044	1.007		1.007	0.137083	0.018792	0.965	0.07975	0.00636
	H4		0.017	0.996	0.979		0.979	0.109083	0.011899	0.817	-0.06825	0.004658
wt	A2	2	0.018	0.93	0.912							
	B2		0.01	0.9	0.89							
	C2		0.011	0.998	0.987	avg	0.869917				0.88525	
	D2		0.017	1.015	0.998	sum			0.264663			0.062644
	E1		0.014	0.884	0.87	div n-1			0.02406			0.005695
	E2		0.015	0.824	0.809	S			0.155114			0.075465
	F1		0.01	0.945	0.935	SE			0.044777			0.021785
	F2		0.021	0.803	0.782	SE_sq			0.002005			0.000475
	G1		0.036	0.899	0.863	Sd			0.049796			
	G2		0.015	0.81	0.795	mean1-mean2			0.015333			
	H1		0.008	0.973	0.965	t			0.307926			
	H2		0.041	0.858	0.817							

T-test Results

Week #	Calculated t-value	Critical value	Calculated >critical	Null Hypothesis
1	0.307926	2.201	No	Accept
2	0.527295	2.201	No	Accept
3	0.183148	2.201	No	Accept
4	1.966624	2.201	No	Accept
5	3.16277	2.201	YES	REJECT
7	0.437061	2.201	No	Accept

Graphical Analysis Results



Conclusions

- Weeks 1-4 & 7 = no significant change
- Week 5 had a significant change between pre read and post growth counts
  - Mutant change in OD > wildtype change in OD
  - Mutation of *ABZ1* gene caused yeast to live longer

What's Next?

- What would week 6 results have looked like?
- Repeated trials needed for clarification
- How does folate level affect other species' lifespans?

References

- Botet J, Mateos L, Revuelta JL, Santos MA. 2007. A Chemogenomic Screening of Sulfanilamide-Hypersensitive Saccharomyces cerevisiae Mutants Uncovers ABZ2, the Gene Encoding a Fungal Aminodeoxychorismate Lyase. Eukaryotic Cell [Internet]. [2007 Sep 14, cited 2015 Mar 8] 6(11): 2102-2111.
- Folic acid in diet [Internet]. St. Elizabeth Healthcare; [2013 Feb 18, cited 2015 Mar 8] . Available from: http://www.stelizabeth.com/ADAM/doc/HIE%20Multimedia/1/002408.htm
- Natural Health Products Ingredients Database [Internet]. Health Canada [cited 2015 Mar 8]. Available from: http://webprod.hc-sc.gc.ca/nhpid-bdipsn/ingredReq.do?id=2932&lang=eng
- Powers III RW, Kaeberlein M, Caldwell SD, Kennedy BR, Fields S. 2006. Extension of chronological life space in yeast by decreased TOR pathway signaling. *Genes & Dev.* 20:174.
- Saccharomyces Genome Database: Cherry JM, Hong EL, Amundsen C, Balakrishnan R, Binkley G, Chan ET, Christie KR, Costanzo MC, Dwight SS, Engel SR, Fisk DG, Hirschman JE, Hitz BC, Karra K, Krieger CJ, Miyasato SR, Nash RS, Park J, Skrzypek MS, Simison M, Weng S, Wong ED (2012) Saccharomyces Genome Database: the genomics resource of budding yeast. Nucleic Acids Res. Jan;40(Database issue):D700-5
- Virk B, Carreia G, Dixon DP, Yeyst I, Jia J, Oberleitner N, Briggs, Z, Hodge E, Edwards R, Ward J, Gems D, Weinkove D. 2012. Excessive folate synthesis limits lifespan in the *C. elegans: E. coli* aging model. *BMC Biology* 10:67.