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Reversal Effects of Probiotic Supplementation on a High Glucose Diet in Caenorhabditis elegans Model

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THE EFFECTS OF PROBIOTICS SUPPLEMENTATION ON HEALTH USING *CAENORHABDITIS ELEGANS* AS A MODEL SYSTEM

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Abstract

With an increasing glycemic index associated with the Western diet, it comes to no surprise that obesity, diabetes, and heart disease are on the rise. Shortened lifespan is associated with these diseases and consumption of a high sugar diet. The benefits probiotics have on shortened lifespan induced by a high glucose diet and the effects of probiotics in combination of cranberry extracts in the model system *Caenorhabditis elegans* were investigated. When *C. elegans* was supplemented with each probiotic strain, lifespan extension was observed. Reversal of glucose induced shortened lifespan and lifespan extension in combination of cranberry extracts was strain dependent.

Introduction

The "Western Diet," prominent among developed nations, often refers to a diet rich in meat proteins and refined sugars. With an increasing glycemic index observed in Western society, it comes to no surprise that obesity, diabetes, and heart disease are on the rise. The Centers for Disease Control and Prevention describes obesity as an epidemic, affecting more than 35% of U.S. citizens. Shortened lifespan and increased susceptibility to pathogens are associated with these diseases and linked to increased consumption of foods high in sugar. In order to reverse these observed effects, the healthful benefits probiotics have on immune system stimulation, restoration of shortened lifespan induced by a high glucose diet, and the beneficial effects of probiotics in combination of cranberry extracts in the model system *Caenorhabditis elegans* were investigated. An exciting benefit of implementing *C. elegans* in such studies is the innate immunological response and aging pathways homology in humans. By targeting these pathways, *C. elegans* provides an economical model system with application in humans. Here, we investigated the effects probiotics have on high glucose diet in *C. elegans*. Consistent with previous studies, when *C. elegans* was supplemented with each probiotic strain, lifespan extension was observed. Interestingly, the reversal of glucose induced shortened lifespan and the extension of lifespan in combination of cranberry extracts was strain dependent. The impact that probiotics have on the reversal of detrimental effects associated with a high glucose diet, including protection against known pathogens and immunological response pathways targeted by probiotics, is currently under investigation.

Specific Aims

1. Determine each probiotic's effect on lifespan
2. Determine if probiotics have the ability to reverse the detrimental effects of a high glucose diet.
3. Determine if the probiotics in combination with cranberry extracts (CBE) further extend lifespan.

Probiotics Used in this Study

Escherichia coli OP50 (control)
Bacillus subtilis
Bacillus coagulans
Lactobacillus fermentum
Lactobacillus plantarum
Lactobacillus delbrueckii

Methods

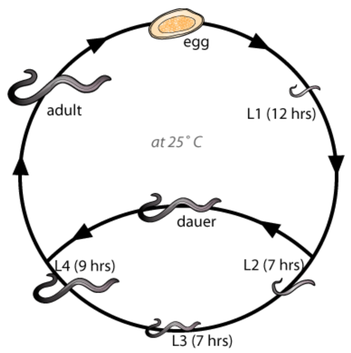


Figure 1. *C. elegans* life cycle at 25°C (artwork from http://www.scq.ubc.ca/genetic-studies-of-aging-and-longevity-in-model-organisms/).

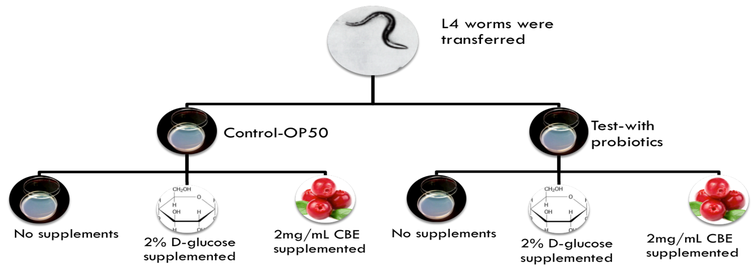
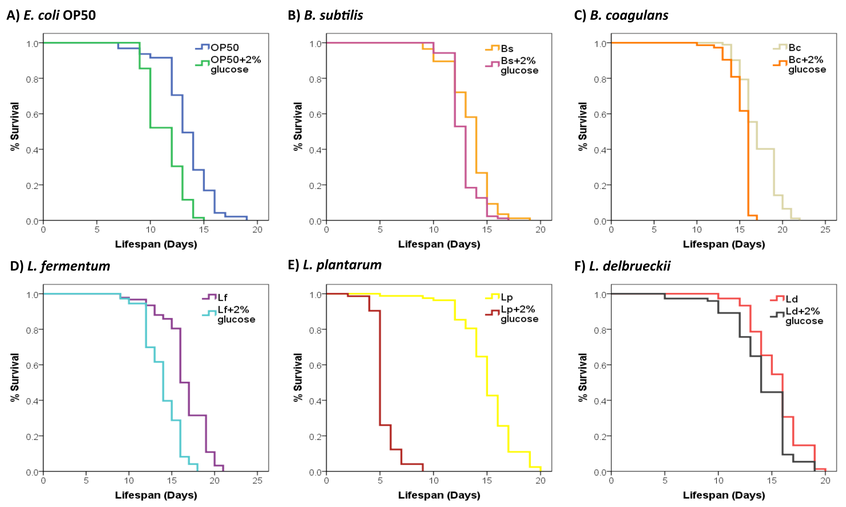


Figure 2. Diagram of the experimental design of lifespan assays carried out at 25°C with different diets.

Results



Diet	Mean±SE (Days)	N	P-value	% Decrease of Lifespan
E. coli OP50	13.442±0.225	95	-	-
OP50+2% glucose	11.333±0.207	69	<0.05	15.70%
B. subtilis	13.477±0.202	86	-	-
Bs+2% glucose	12.759±0.138	87	<0.05	5.30%
B. coagulans	17.261±0.221	92	-	-
Bc+2% glucose	15.301±0.142	73	<0.05	11.39%
L. fermentum	16.663±0.262	92	-	-
Lf+2% glucose	13.986±0.240	73	<0.05	16.10%
L. plantarum	15.085±0.273	82	-	-
Lp+2% glucose	5.342±0.130	73	<0.05	64.60%
L. delbrueckii	15.48±0.256	75	-	-
Ld+2% glucose	14.135±0.318	74	<0.05	8.70%

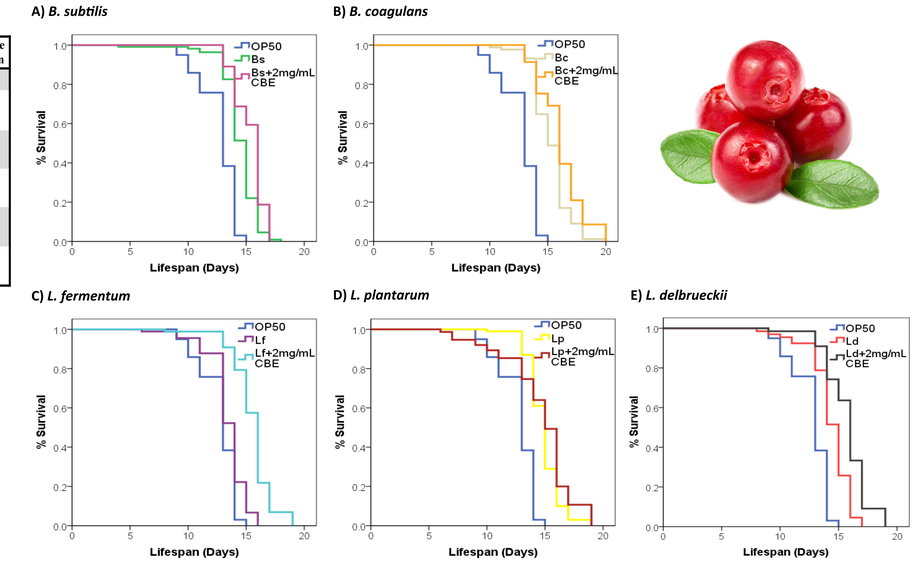
Table 1. Lifespan of N2 worms on high glucose diet and probiotics at 25°C. N2 lifespan was shortened with all strains when supplemented with glucose. Lifespan restoration was observed in *Lactobacillus delbrueckii*, *Bacillus subtilis*, and *Bacillus coagulans*. Worms fed *Lactobacillus plantarum* exhibited an extreme decrease in lifespan when supplemented with 2% D-glucose. Data shown are representative of one round of lifespan assays.

Figure 3. Lifespan curves of N2 worms fed on each probiotic with or without 2% glucose supplemented into diet at 25°C.

Diet	Mean±SE (Days)	N	P-value	% Increase of Lifespan
E. coli OP50	12.737±0.459	99	-	-
B. subtilis	14.468±0.369	109	<0.05	13.6%
B.s.+2mg/mL CBE	15.359±0.106	64	<0.05	20.6%
B. coagulans	15.295±0.181	88	<0.05	20.1%
B.c.+2mg/mL CBE	16.136±0.130	81	<0.05	26.7%
L. fermentum	13.44±0.511	90	<0.05	5.5%
L.f.+2mg/mL CBE	15.575±0.320	87	<0.05	22.3%
L. plantarum	14.9±0.194	100	<0.05	17%
L.p.+2mg/mL CBE	14.693±0.300	75	<0.05	15.4%
L. delbrueckii	14.364±0.459	66	<0.05	12.8%
L.d.+2mg/mL CBE	15.742±0.304	66	<0.05	23.6%

Table 2. Lifespan of N2 worms fed on probiotics and CBE diet at 25°C. N2 lifespan was increased with all strains when supplemented with CBE except L. planatarum. Lifespan extension was observed in *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Bacillus subtilis*, and *Bacillus coagulans*. Data shown are representative of one round of lifespan assays.

Figure 4. N2 lifespan curves carried out at 25°C with and without 2mg/mL CBE and probiotics.



Conclusion

1. Consistent with previous research, the probiotic strains tested extended lifespan.
2. Consistent with previous research, 2% D-glucose decreased N2 worms' lifespan.
3. Probiotics alleviated the effects of a high glucose diet in *C. elegans* in a strain dependent manner.
4. *L. plantarum* enhanced the detrimental effects of a high glucose diet in *C. elegans*, resulting in a drastic reduction of lifespan.
5. Probiotics in combination with cranberry extracts further extended lifespan in a strain dependent manner.

Future Directions & Significance

Insulin, a hormone involved in regulating metabolism and absorption of glucose in mammals, and the homolog IGF-1 have been shown to regulate lifespan in many organisms and is involved in other cellular processes such as stress response. The FOXO transcription factor DAF-16 in the insulin/IGF-1 signaling pathway and decreased activity of heat shock transcription factor HSF-1 reduced the rate of tissue aging, resulting in increased lifespan. The DAF-16 and HSF-1 transcription factor may be inhibited by a high-glucose diet. A downstream gene encoding a glycerol channel *aqp-1* was also involved in lifespan reduction induced by a high glucose diet. In mammals, aquaporin glycerol channels are repressed by insulin. We intend to target these pathways to determine their involvement in probiotic mediated restoration of lifespan.

Our findings may also have implications in prevention and/or cure of obesity related diseases such as type 2 diabetes, tumor formation and heart disease. Studies could be further carried out in a higher organism such as mice. Because of the pathway homology including insulin/IGF-1-mediated signaling (IIS) pathway, p38 kinase pathway, and other immunological/stress response pathways between *C. elegans* and humans, these findings may be of high human health importance. In addition these findings may lead to relatively inexpensive and highly accessible prevention or treatment for infection.

Acknowledgments

Cranberry extracts were obtained from DECAS.

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