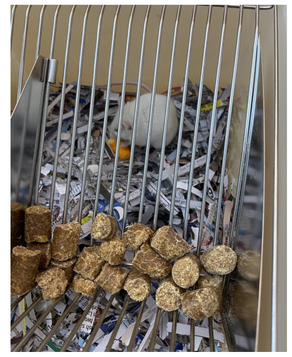
Effects of the Probiotic *Saccharomyces boulardii* on *Escherichia coli* in Fecal Samples of *Mus musculus*

Shelby Joy

Department of Sciences & Mathematics, Saint Mary-of-the-Woods College, Saint Mary-of-the-Woods, IN 47876



Introduction

Escherichia coli is the most abundant facultative anaerobe composing the normal flora of the gastrointestinal (GI) tract. To enhance and maintain the GI tract people often take probiotic supplements. *Saccharomyces boulardii* is a known probiotic yeast that is believed to improve the gut immune response and the intestinal barrier but the mechanisms underlying these protective actions are not well understood (Zhou et al., 2018). For this research, the probiotic yeast *Saccharomyces boulardii* was tested to see if they work by enhancing the numbers of *E. coli*.

Fig. 1. The *Saccharomyces boulardii* supplement used in this research.

Hypotheses

H_1: The treatment group will have more *E. coli* present in the fecal samples than the control group.

H_0: There will be no statistical difference in the abundance of *E.coli* in the fecal samples.

Materials and Methods

Group	Supplement
Control	Cheeto
Treatment	Cheeto with Probiotic

Table 1. A summary of experimental groups and the daily supplements.

Treatment: The Animal Equivalent Dose (AED) conversion method was used to calculate the correct dose for each mouse based on their weight (Nair & Jacob, 2016).

Fecal Sample: Three samples were collected per mouse. Each sample containing one pellet. Phosphate Buffer Saline was used to perform a ten-fold dilution on each sample. Samples were vortexed before and after each dilution. The 1:100 dilution was then plated for each sample.

After incubating these original plates for 48 hours, a master plate was made. This master plate was then incubated. After incubation, testing could be performed on the colonies of the master plate while maintaining the plate.

Fig. 2. A plate created from a fecal sample diluted by a ratio of 1:100.

Fig. 3. A master plate created from the plate of a diluted fecal sample.

Materials and Methods Continued

Isolating Probable *E. coli*: *E. coli* is one of few microorganisms in the GI tract that is oxidase negative and indole positive. To determine which colonies in the samples fit this criteria, the oxidase test as well as the indole test was performed.

To determine the total number of *E. coli* in each sample, each colony on the master plates were analyzed. Those colonies that were oxidase negative and indole positive were likely *E. coli* and used to determine the total number of *E. coli* in each sample.

Fig. 4. Oxidase Test. The purple indicates oxidase positive and the yellow/clear indicates oxidase negative.

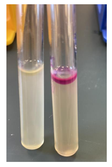


Fig. 5. Indole Test of two colonies. The yellow ring indicates indole negative, and the reddish ring indicates indole positive.

Results & Discussion

During analysis, the percentage of probable *E.coli* was determined for each sample. From this the average percentage of probable *E.coli* was determined for each mouse.

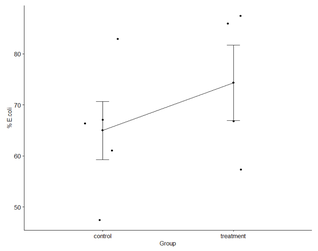
Welch Two Sample T-test

t	df	P-value
-1.0041	6.0451	.8231

Table.2. Summary of the Welch T-test results

A Welch two-sample t-test was performed to determine if there was a significant difference in the percentage of *E. coli* between the control group and the treatment group. This specific t-test was chosen because the groups had unequal variance. This was supported by an F test.

The Percentage of Probable *E.coli* in the Control Group vs. the Treatment Group



Graph.1. The lines on this graph indicates the 95% confidence interval for the actual mean of each group. The dots represent individual sample percentages and the dots with a line connecting them is the sample means for each group.

I then performed a False Positive Risk Analysis (FPR). This test shows the probability that your results are due to chance (Wasserstein et al., 2019). My FPR=.607 or 60% using the Selke-Berger Approach.

$$FPR = \frac{1}{1 + L_{10}\frac{\mathrm{P}(H_1)}{1 - \mathrm{P}(H_1)}}$$

Equation. 1. This equation is used to calculate the False Positive Risk.

Conclusions

The false positive risk that was calculated was 60%. This means there is a 60% chance that the bacteria was produced by pure chance.

The Welch Two Sample T-test indicated that there was not enough evidence to determine if there was more *E.coli* in the treatment group than the control group.

With these results, further research is needed to determine the relationship between *S. boulardii* and *E. coli*.

Future Work

- Test other *S. boulardii* supplements against specific GI diseases.
- Test *S. boulardii* affects against other microorganisms in the GI tract.
- Work to identify how *S. boulardii* stimulates immune responses in sick patients.

Literature cited

Zhou, H., Zhang, H.-J., Guan, L., Zhang, Y.-N., Li, Y., & Sun, M.-J. (2018). Mechanism and therapeutic effects of Saccharomyces boulardii on experimental colitis in mice. Molecular Medicine Reports, 18(6), 5652–5662. https://doi.org/10.3892/mmr.2018.9612

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Acknowledgments

The Saint Mary-of-the-Woods College Department of Sciences and Mathematics, including faculty and students.