The Investigation of the Effect of Iron, Zinc and Biotin on the Metabolism of *Saccharomyces boulardii* Cells

Extended Essay (Biology)

Session: May 2015

Candidate Name: Ege ÜNLÜ

Candidate Number: D11290107

Centre Name: TED Ankara College High School, Ankara, TURKEY

Centre Number: 1129

Supervisor: Sevim Saral

Word Count: 4233

ABSTRACT

Probiotics are beneficial microorganisms that are consumed to introduce their effects to their host. The focus of this study is concentrated upon *Saccharomyces boulardii*, a probiotic yeast species, which has many medical uses because Saccharomyces boulardii is thought to display antitoxin, antimicrobial, anti-inflammatory effects and increased immune response. It is mainly used as a medicine against diarrhea and marketed under the name of Reflor®. Thus the study focuses on factors that would help to increase the potency of the drug Reflor® by boosting the metabolism of S. boulardii. The aim of this investigation is examining the effects of iron, zinc and biotin on the metabolic rate of S. boulardii colonies by collecting the carbondioxide gas evolved, through the metabolic pathway of ethanol fermantation of S.boulardii cells, in a U-tube system. As all other factors that could potentially effect S. boulardii metabolism and growth are controlled, the effect of different substances; iron, zinc and biotin are effectively investigated by measuring the difference between heights of water colons changed due to anaerobic respiration of S. boulardii in equal time intervals.

The mean value for difference of heights of water colons for controlled, iron, zinc, biotin and iron+zinc+biotin groups are respectively; 0.90, 1.18, 1.16, 0.98, 1.40 cm. According to the obtained data, calculated mean values and statistical analysis; in this investigation the the hypothesis is supported by evidence. S. boulardii reacted differently in the presence of different minerals and vitamins and showed the highest metabolic rate and growth in the presence of iron and zinc ions. Biotin, on its own, is observed to be the least effective additive, however the combination of all three substances boosted the metabolism of S. boulardii significantly. Overall, the results of this study advocated the hypothesis that iron and zinc is much needed for the good growth and increased potency of S. boulardii, while biotin has also supported the good growth of yeast.

Word Count: 315

CONTENTS

INTRODUCTION

The nature of disease causing organisms and their unseen kingdom have always been a topic of interest for me. When I heard diarrhea was the second most common cause of deaths in children younger than five, with a general frequency of billions of cases around the globe[1]; I was particularly interested and felt compelled to investigate the disease further. The most common reason of the disease is outlined as an infection of the intestines due to either a virus, bacteria, parasite, or a condition known as gastroenteritis. These infections are often acquired by contaminated food and water and lack of sanitation and hygiene. Thus this easily treated disease, takes millions of lives in developing countries.[2] As to my chance, when I personally contacted the diseases, I took one single tablet of Reflor® (250 mg) and it singlehandedly solved my problem right away. Then I was amazed by the potency of this drug and simply interested by the mechanism behind its action. As one tiny tablet regulated my entire digestive system which consisted of metres of intestines.

Diarrhea is the condition of having at least three loose or liquid bowel movements each day. It often lasts for a few days and can result in dehydration due to fluid loss. Prevention of infectious diarrhea is by improved sanitation, clean drinking water, and hand washing. Yet these basic human needs are not satisfied in many developing countries which results in millions of deaths every year. These treatments have been estimated to have saved 50 million children in the past 25 years.[3]

1 ,2 "Diarrhoeal disease Fact sheet N°330". World Health Organization. April 2013. Retrieved 18 June 2014.

3 "whqlibdoc.who.int" (PDF). World Health Organization.

The fatality of diarrhea is mostly dependent on the financial development levels of nations. It is treatable disease with only proper nutrition however, this kind of proper nutrition is often very hard to be sustained in long term for many countries. Cheap and effective medication against many kinds of diarrhea is though available. Reflor tablets cured me almost instantly; and when I checked the prospectus, I have seen that the active ingredient of the drug was a biologically active, lyophilized species of fungi. It was named as *Saccharomyces boulardii.*

Saccharomyces boulardii is a tropical strain of yeast first isolated from lychee and mangosteen fruit in 1923 by French scientist Henri Boulard.[4] It is related to, but distinct from, *Saccharomyces cerevisiae* in several taxonomic, metabolic, and genetic properties. *S. boulardii* is sometimes used as a probiotic with the purpose of introducing beneficial active cultures into the large and small intestine, as well as conferring protection against pathogenic microorganisms in the host.[5][6][7] So *Saccharomyces boulardii* acts as a probiotic organism that provides ultimate protection against diarrhea.

4 Malgoire JY, Bertout S, Renaud F, Bastide JM, Mallié M (2005). "Typing of Saccharomyces cerevisiae clinical strains by using microsatellite sequence polymorphism". J. Clin. Microbiol.

5 Rajkowska, Katarzyna; et al. (April 2012). "Probiotic Activity of Saccharomyces cerevisiae var. boulardii Against Human Pathogens".Food Technology and Biotechnology 50: 230–236. Retrieved 18 January 2014.

6 Toma, Malda Maija; et al. (June 2005). "Effect of Probiotic Yeast on Genotoxicity". Food Technology and Biotechnology 43: 301–305. Retrieved 18 January 2014.

7 Soccol, Carlos Ricardo; et al. (June 2010). "The Potential of Probiotics: A Review". Food Technology and Biotechnology 48: 413–434. Retrieved 18 January 2014.

Sacchormyces boulardii grows at the unusually high temperature of 37°C.[8] The fact that *Saccharomyces Boulardii* prefers anaerobic conditions and an optimum temperature of 37°C along with its non-pathogenic properties and many beneficial aspects make it a great probiotic and a living defence mechanism against other disease causing pathogens in intestines. *Saccharomyces boulardii* is thought to display antitoxin, antimicrobial, anti-inflammatory effects and increased immune response.

After Reflor is ingested orally, *Sachharomyces boulardii* yeasts form a temporary colony in intestines and regulate intestinal activity in a short time. In an, "in vivo" system yeasts can provide all of their nutritional requirements from digested monomers in human guts. Main nutritional requirements of yeasts is sugar to fermantate, nitrogen source mainly ammonia, and metals like iron, calcium, zinc and magnesium. Most strains are prototrophic to vitamins, so biotin and pantothenate are often required for full growth and function.

Saying that the intestines of a person that is suffering from diarrhea has partially lost their function and many of the mutualistic bacteria is washed away from the intestines. Such a person would also be suffering unsufficient mineral and vitamin uptake. This would also impair the metabolic activity of the ingested *Sacchormyces boulardii*. I have personally wondered if there was a significant correlation between the metabolic rates of the *Saccharomyces Boulardii* yeast colonies and the mineral and vitamin concentrations of the environment that they thrive on. If I could figure out such relationship than it would be

8 Li Ping Du et al., 2011, Advanced Materials Research, 343-344, 594

reasonable that any of those vitamins or minerals would create a synergistic effect with Reflor; and for instance an extra Iron tablet along with Reflor would be better for a more precise and efficient recovery. Outlining the existence of such an effect is the aim of this investigation.

Iron, Zinc and Biotin of different concentrations will be tested upon samples of *Saccharomyces boulardii*, and changes in metabolic patterns of the yeast will be observed. Evolved carbondioxide gas will be collected in u-tubes as an indicator of metabolic rates of the yeast colonies as *Sacchormyces boulardii* relies on ethanol fermantation to produce the suffiecient energy for its metabolic activities. Iron, Zinc and Biotin are especially chosen as they are the three of most commonly used nutritional back up products by the general population and have ignorable toxicity values along with high solubility.

Does the presence of Iron ions, Zinc ions and dissolved biotin in the environment, affect the metabolic rates of the *Saccharomyces boulardii* colonies under identical growth conditions if evolved carbondioxide gas is collected in an U-tube system as an indicator of metabolic rate and growth?

This research question, along with the detailed biological aspects of this yeast strain will be discussed throughout the paper.

HYPOTHESIS

Intestines of a human being is one of the most nutrient rich parts of his body. In the case of diarrhea, constant bowel movements and loss of appetite turns intestines into nutrient depleted parts of the body. If the *Sacchormyces boulardii* colonies are to survive and thrive in this kind of an environment, they would need more than sugar to anaerobically produce energy. In order to help with the full growth of this specific strain of yeast that we would make the use its probiotic benefits, some minerals and vitamins might be needed to externally introduced to the environment.

Introduction of Iron, Zinc and Biotin are all expected to act on the metabolic rates of *Saccharomyces boulardii* colonies. The presence of sufficient concentrations of these minerals and vitamin is thought to be not necessary for the survival of the yeast colonies but presence of these factors may accelerate yeasts' metabolism and increase the potency of the medication. So there is a relationship between the concentrations of dissolved Iron, Zinc and Biotin and the metabolic rates (evolved CO2 gas) of *Saccharomyces boulardii* fungi colonies that specific minerals are given to.

If the effects of Iron, Zinc or Biotin alone are to be expected; Zinc and Iron concentrations should be much more limiting factors on the growth and metabolism of *Saccharomyces boulardii*. This is because, the vitamin requirements of living bodies are much less then their mineral requirements. Thus Iron and Zinc concentrations would have much more distinct and significant effects on metabolic rates of Saccharomyces boulardii cells. If all of these minerals and vitamins are to be combined in one environmental systems, that system would accomodate the yeast colonies with highest metabolic rates.

METHOD DEVELOPMENT AND PLANNING:

To test the research question "**Does the presence of Iron ions, Zinc ions and dissolved biotin in the environment, affect the metabolic rates of the *Saccharomyces boulardii* colonies under identical growth conditions if evolved carbondioxide gas is collected in an U-tube system as an indicator of metabolic rate?",** Iron, Zinc and Biotin (of sufficient concentrations) should be administrated, on identical Saccharomyces boulardii cells, in different groups.

I investigated this research question at TED Ankara College Foundation Private High School and acquired laboratory access from Sevim Saral, my supervisor and a biology teacher at TED Ankara College. The equipment and the solutions used in the experiment were supplied by the Department of Biology.

In order to observe the effect of different minerals and vitamins on the metabolism of *Saccharomyces boulardii*, one should first establish the essential growth factors that are needed by the yeast. The essential growth factors are optimum temperature, pH, nitrogen source and fermentable carbonhydrates. Optimum temperature for Saccharomyces Boulardii is 37°C, this is a remarkable advantage for its use in human intestines, as many other yeast strains have an optimum temperature of 25-30°C. Temperature of all experimental groups will be stabilized at 37°C by the use of waterbaths. *Saccharomyces Boulardii* is very tolerant to pH changes and can survive properly except extremely basic or acidic environments. Its optimum pH range is stated as 4.5-6.5.[9] Thus a buffer solution of 10 mL are put to 200 mL of water in

9 J Gen Appl Microbiol. 2008 Aug;54(4):221-7.

every experimental group. The buffer solution initially had a pH of 5.0 and consisted of citric acid and sodium hydroxide. As 5 mL 1M of ammonia is added to every group as a nitrogen source for yeasts, pH in all groups are stabilized at 5.2. The most important nutritional requirement of the yeast is an energy source and glucose is the most adequate source. Optimum glucose concentration for best *S. boulardii* growth is 100 g/L thus 20 grams of glucose are dissolved in 200 mL of water in every system. *Saccharomyces boulardii* prefers fermentation over respiration 98 to 2%. Thus anaerobic conditions are fit for *S. boulardii*. They are precipitated to the bottom of the flask that is full of water in order to prevent the direct contact with the oxygen rich air. As all of the essential growth factors are kept constant in aforementioned ways, water baths, buffer solutions, glucose and ammonia, together with distilled water and conical flasks are needed.

The independent variables are the minerals and vitamins that are dissolved in conical flasks. A controlled group is established so that data from other experimental groups could be compared with a group with no iron, zinc nor biotin. As the optimum conditions for *S. boulardii* are being reflected, conditions inside the human intestines are also mimicked. A synergistic nutritional supply is being investigated, already used Iron, Zinc and Biotin nutritional backup tablets are used in this investigation. For Iron, Feramat® capsules; for zinc Zinco® tablets; and for biotin MedobioHtin® tablets are selected. Healthy dose to take up at once is one capsul for each of these medications for human beings. In the light of these facts, for group 2, one Feramat® capsule is broken and Ferrum Fumarat that is equivalent to 100 grams of Iron is dissolved in water. For group 3, Zinc Sulfate Monohydrate equivalent to 50 mg of Zinc is dissolved. For group 4, one tablet of MedobioHtin tablet is dissolved in water which means 5 mg of Biotin. Finally for group 5, all tablets are dissolved which means a mixture of 100 mg of

Iron, 50 mg of Zinc and 5 mg of Biotin. Even though the concentrations of added minerals and vitamin B7 are different, these are the healthy concentrations of healthy daily consumption for human body so using already existing soluable drugs was the best choice available.

The metabolic fermantation pathway of *S. boulardii* is ethanol fermantation which means a glucose is broken down to ethanol and carbon dioxide gas to produce cellular energy. As gas molecules are evolved through their metabolic pathway, collection of this carbondioxide gas in a U-tube system and taking necessary measurements would give necessary data for a common understanding of metabolic rates of *S. boulardii*. So that we can understand which type of mineral or vitamin is most needed to increase the potency of Reflor®. Basically as gas is evolved, through a plastic pipe gase will apply pressure to one end of the water filled U-tube so water will rise in the other side. The difference in heights of water colons would be measured in milimeters every two hours for a total of ten hours. pH measurements are not required as buffer solutions is more than enough to stabilize the pH as initially measured value of 5.2.

1 sachet of Reflor® which contains 250 mg of *Saccharomyces boulardii* shall be added to every group after everything was ready.

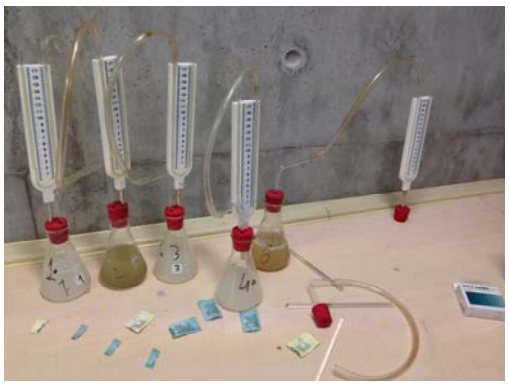
METHOD:

Materials Used in the Experiment

- 25 conical flasks
- 5 L Distilled Water
- 1 Water Bath
- 5 U-tubes
- 5 plastic pipes
- 5 corks with 2 holes
- 125 mL 1M ammonia solution
- 500 grams of glucose
- 25 Reflor®(250 mg) sachets
- 10 Zinco® capsules
- 10 Feramat® capsules
- 10 MedobioHtin® tablets
- 10 stirrers
- 250 mL pH 5 citric acid/sodium hydroxide buffer solution
- 2 pH meters
- 5 glass pipes
- 1 knife
- 1 balance
- 50 plates
- 10 graduated cylinders

Procedure:

1) Fill 5 conical flasks with 200 mL of distilled water.
2) Use the weighing machine to prepare 5 plates of 20 grams of glucose.
3) Add 20 grams of glucose to every flask.
4) Pour 10 mL of buffer solution (that is already prepared) to every flask by using graduated cylinders.
5) Pour 5 mL of 1 M ammonia to every flask.
6) Cut open a Feramat® capsule and put the insides to a plate and then add to the flask 2 and 5.
7) Cut open a Zinco® capsule and put the insides to a plate then add to the flask 3 and 5.
8) Dissolve a MedobioHtin tablet in flask 4 and 5.
9) Place all flasks in water bath and set the temperature to 37°C.
10) Stir the solutions in all flasks until all substances are dissolved.
11) Take a pH measurement from every flask.
12) Empty 1 sachet of Reflor® to every flask. Be sure to empty all the sachet.
13) Close the flasks with corks and set up the U-tube system.
14) Measure the height difference in water colons of U-tube in every two hours for ten hours.



Picture A and B: Pictures of experimental design showing all five experimental groups.

RESULTS:

Time (Hours ±0,1)	Trials	Difference in Water Colon Heights in U-tube (cm±0,01)					Temperature of the system (°C±0,1)	pH of solution (±0,1)	Volume of dissolved 1 M Ammonia (±0,1 mL)	Mass of dissolved glucose (±0,001 g)	Volume of Added Buffer Solution (±0,1 mL)
		Controlled Group	Iron Group	Zinc Group	Biotin Group	Zinc+Iron+Biotin Group					
	1	0,00	0,00	0,00	0,00	0,00					
	2	0,00	0,00	0,00	0,00	0,00					
	3	0,00	0,00	0,00	0,00	0,00					
	4	0,00	0,00	0,00	0,00	0,00					
0,0	5	0,00	0,00	0,00	0,00	0,00					
	1	0,1	0,1	0,2	0,1	0,3					
	2	0,0	0,3	0,1	0,1	0,2					
	3	0,1	0,3	0,2	0,2	0,2					
	4	0,1	0,2	0,3	0,1	0,2					
2,0	5	0,1	0,2	0,2	0,1	0,3					
	1	0,2	0,3	0,5	0,3	0,7					
	2	0,2	0,5	0,3	0,3	0,5					
	3	0,3	0,6	0,4	0,4	0,4					
	4	0,3	0,4	0,4	0,2	0,5					
4,0	5	0,2	0,4	0,3	0,2	0,6					
	1	0,4	0,6	0,8	0,6	1,0					
	2	0,5	0,7	0,6	0,5	0,8					
	3	0,6	0,8	0,7	0,6	0,7	37,0	5,2	5,0	20,000	10,0
	4	0,6	0,7	0,7	0,4	0,8					
6,0	5	0,5	0,8	0,6	0,5	0,9					
	1	0,6	0,9	1,1	0,8	1,3					
	2	0,7	0,9	0,9	0,7	1,0					
	3	0,8	1,0	0,9	0,9	1,0					
	4	0,9	1,0	1,0	0,6	1,1					
8,0	5	0,7	1,1	0,9	0,7	1,2					
	1	0,8	1,2	1,3	1,0	1,6					
	2	0,9	1,1	1,0	1,0	1,2					
	3	0,9	1,2	1,1	1,2	1,3					
	4	1,0	1,1	1,2	0,8	1,4					
10,0	5	0,9	1,3	1,2	0,9	1,5					

Table 1:The difference in water colon heights in U-tube according to time for every experimental group and trial along with controlled variables of temperature of system, pH of solution, volume of dissolved ammonia, mass of dissolved glucose and volume of added buffer solution with appropriate units and uncertainties.

During the experiment, one can realize some changes with his senses. These qualitative data can be summarized as:

- The production of bubbles and foams on the top of solutions, indicating gas is evolved throughout the experiment which is most probably carbondioxide due to fermantation pathway.
- A greenish dark color is observed when the Feramat® capsule dissolved in water. This is probably a result of artificial dyes that are used in capsules or it might be directly caused by the Ferrum Fumarat (Iron compound in Feramat®).
- After the experiment was completed and flasks are being washed, a distinctive rotten smell is detected. This might be caused by nitrogen containing metabolic wastes of S. boulardii.

Using the Microsoft Excel 2007 program, one can determine the statistical relationship between the ascorbic acid concentration and the different layers of citruses. The following formulas are used to obtain the values in Table 6.

Mean:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

where;

n is the largest number of trials (5 for this experiment)

x_i is The difference in water colon heights in U-tube

Standard Deviation:

$$\sigma = \sqrt{\frac{\Sigma\ (x - \bar{x})^2}{n}}$$

where;

n is the largest number of trials (5 for this experiment)

x_i is The difference in water colon heights in U-tube

$\bar{x}$ is the mean value of the corresponding group/data

Standard Error:

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

where;

n is the largest number of trials (5 for this experiment)

x_i is The difference in water colon heights in U-tube

σ is the Standard deviation of the corresponding group/data

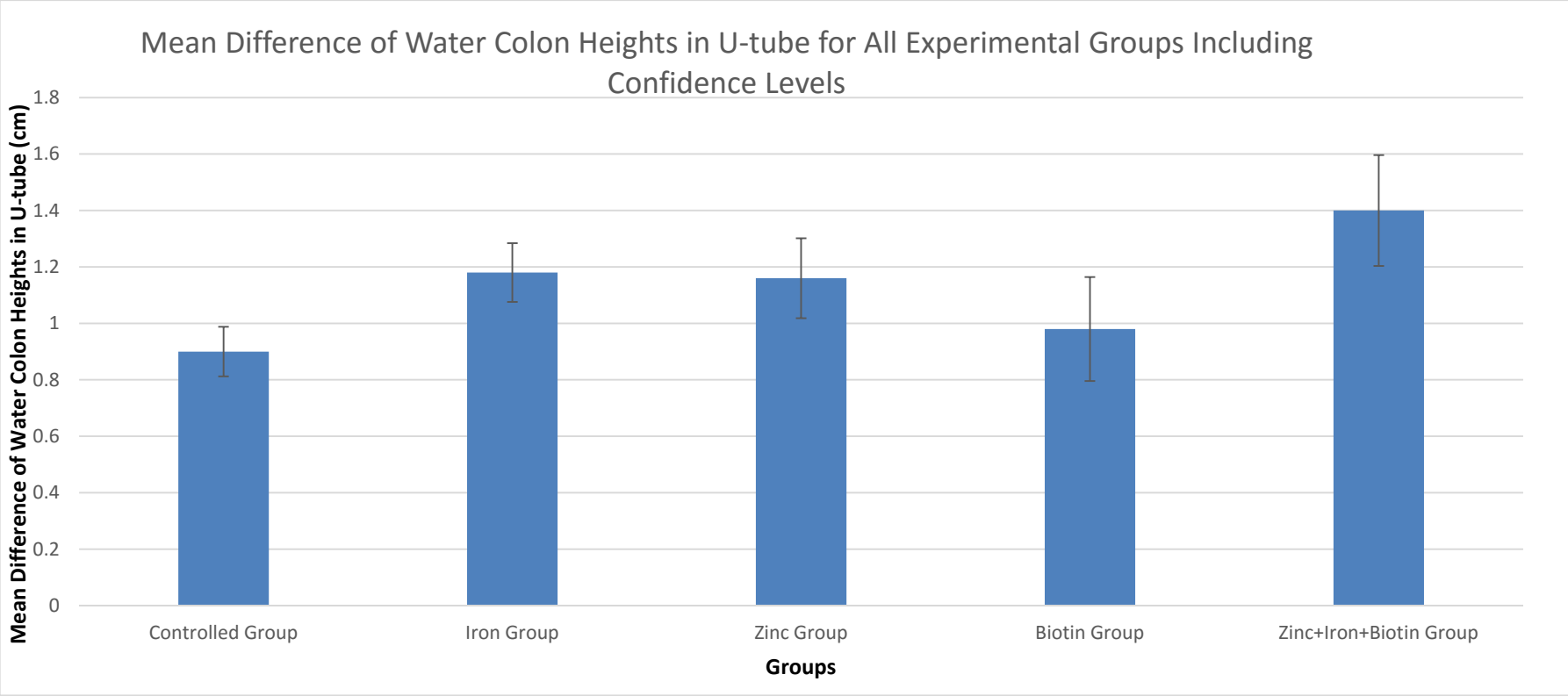
Statistical Analysis					
Groups	Controlled Group	Iron Group	Zinc Group	Biotin Group	Zinc+Iron+Biotin Group
Mean	0,90	1,18	1,16	0,98	1,40
Median	0,90	1,20	1,20	1,00	1,40
Range	0,20	0,20	0,30	0,40	0,40
Variance	0,01	0,01	0,01	0,02	0,02
Standard Deviation	0,07	0,08	0,11	0,15	0,16
Standard Error	0,03	0,04	0,05	0,07	0,07

Table 2: The table of mean, median, range, variance, SD, SE calculations for the independent variable of difference in water colon heights in gas collecting apparatus due to release of carbondioxide gas from *S. Boulardii* cells for Controlled Group, Iron Group, Zinc Group, Biotin Group and Zinc+Iron+Biotin Group for the time of Hour 10,0 from the data of Table 1.

Statistical analysis is conducted for only the last measurement as the final measurement gives all necessary information in a collective manner. Evolution of carbondioxide through time means too little for statistical analysis and for the general aim of the investigation which is targeting to observe the effects of different nutrient supplements. Hour 10,0 is specifically chosen for statistical analysis as enough time has passed for every system to stabilize thus measurements at Hour 10,0 give the most accurate and precise data.

	Controlled Group	*Iron Group*	*Zinc Group*	*Biotin Group*	*Zinc+Iron+Biotin Group*
Confidence Level(95,0%)	0,09	0,10	0,14	0,18	0,20
Mean (cm)	0,90	1,18	1,16	0,98	1,40

Table 3: Confidence level(95,0%) results obtained from the data in Table 1 for Hour 10,0. The mean values are also shown for data Table 1 at Hour 10,0.



Graph 2: Mean Difference in Water Colon Heights in U-tube due to carbondioxide evolution from S. Boulardii cells for all experimental groups at Hour 10,0 including confidence levels as error bars which is drawn using the data in Table 7.

Anova: Single Factor

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,7576	4	0,1894	13,15277778	**2,07249E-05**	2,866081402
Within Groups	0,288	20	0,0144			
Total	1,0456	24				

Table 4: The Anova: Single Factor results obtained from the data in Table 1 for Hour 10,0. The P-value being numerically smaller than the alpha value of 0,05 indicates the null hypothesis is rejected and the alternative hypothesis is accepted.

Result of Anova:

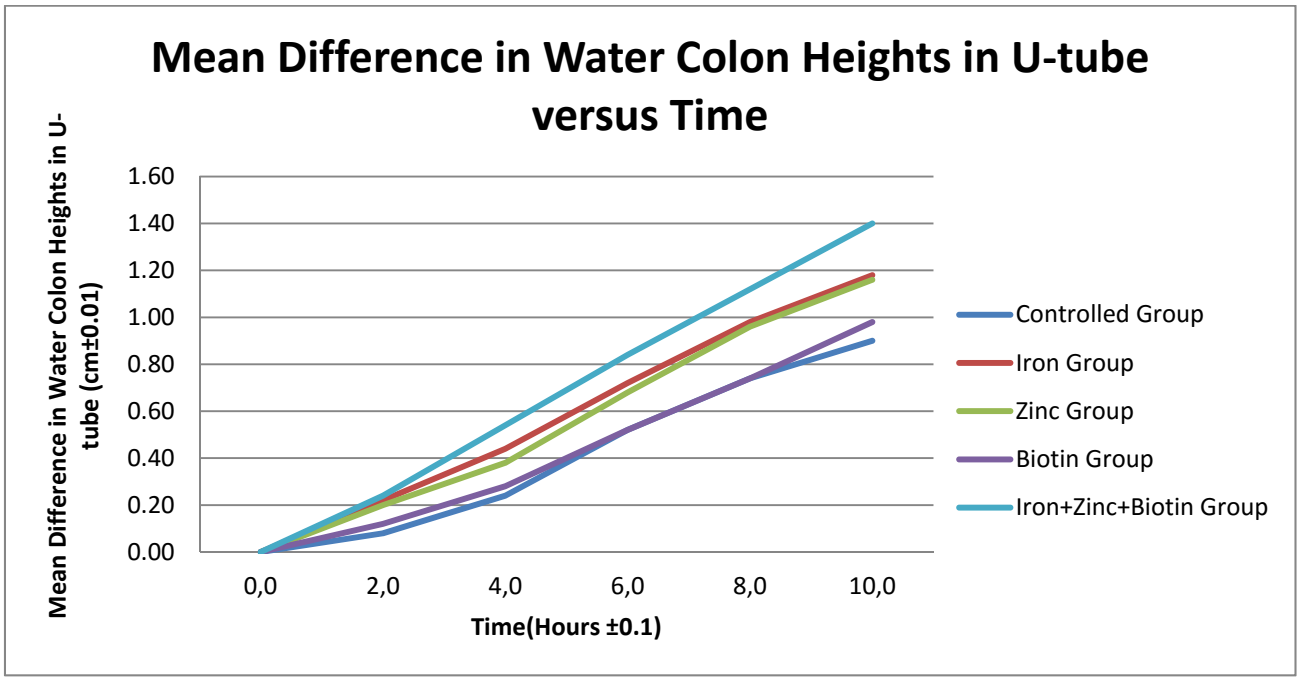
Null Hypothesis: There is **not** a statistically significant mean difference between heights of water column in gas collecting apparatus due to carbondioxide release by the anaerobic ethanol respiration of S. Boulardii when it is grown in media with different nutrient supplements.

Alternative Hypothesis: There is a statistically significant mean difference between heights of water column in gas collecting apparatus due to carbondioxide release by the anaerobic ethanol respiration of S. Boulardii when it is grown in media with different nutrient supplements.

P=2,07249E-05 < α (0,05) thus null hypothesis is rejected and alternative hypothesis is accepted.

Mean Difference in Water Colon Heights in U-tube (cm±0,01)						
Groups	Time(Hours ±0,1)					
	0,0	2,0	4,0	6,0	8,0	10,0
Controlled	0,00	0,08	0,24	0,52	0,74	0,90
Iron	0,00	0,22	0,44	0,72	0,98	1,18
Zinc	0,00	0,20	0,38	0,68	0,96	1,16
Biotin	0,00	0,12	0,28	0,52	0,74	0,98
Iron+Zinc+Biotin	0,00	0,24	0,54	0,84	1,12	1,40

Table 5: The mean difference in water colon heights in u-tube due to carbondioxide evolution from S. boulardii cells metabolic pathway of ethanol fermantation(cm± 0,01) versus time (hours ± 0,1) for every experimental group.



Graph 3: The mean difference in water colon heights in U-tube (cm± 0,01) versus time (hours ± 0,1) for every experimental group is depicted as a line graph according to data in Table 8. General trends for metabolic activities of different groups can be observed from the lines as the yeast colonies in Iron+Zinc+Biotin Group practice the fastest metabolism among other experimentally observed groups.

CONCLUSION AND EVALUATION:

In this experiment, the effect of dissolved Iron, Zinc and Biotin (in the growth environment) on the cellular metabolism rates of probiotic Saccharomyces Boulardii cells was investigated. Different experimental groups for Iron, Zinc and Biotin were prepared along with a controlled group that had no additives of investigated substances and another group that contained all investigated substances including Iron, Zinc and Biotin. Additives are used in different concentrations as their healthy dose of intake for human beings were all different and they were added into identical growth environments of identical *Saccharomyces boulardii* cells to observe the effect of different minerals and vitamins on the metabolic rates of *Saccharomyces boulardii* cells. What was aimed in the overall investigation was finding out the most synergistic mineral or vitamin to help improve the metabolism of probiotic *Saccharomyces boulardii*. Thus the medical probiotic could be used with higher potency.

The investigated substances of iron, zinc and biotin are all obtained from water soluble tablets and pills. Iron and zinc pills were opened and insides were put dissolved in different experimental systems. Biotin tablet was water soluble at all, as it had no outer shell and it was directly dissolved in another experimental system. All tablets included required amounts of daily intake of the mineral or vitamin for a human being and this corresponded to ferrum fumarat equivalent to 100 grams of Iron; zinc sulfate monohydrate equivalent to 50 mg of zinc; one tablet of MedobioHtin® tablet equivalent to 5 mg of biotin. Many other limiting factors on the growth and metabolism of *Saccharomyces boulardii* is hold under control as controlled variables such as pH maintained at 5,2 via addition of buffer solution, temperature

at 37,0°C via waterbath, mass of dissolved glucose was constant of 20,000 grams and 5 ml of 1 M ammonia is added as nitrogen source to all systems. As ethanol fermantation was the preferred metabolic pathway of energy production for Saccharomyces boulardii, anerobic conditions were provided as yeast cells are held in oxygen low distilled water solutions. Carbondioxide output as a metabolic output of *Saccharomyces boulardii* is used as an indicator of metabolic rate as gas is collected in a U-tube and difference in water colon heights of U-tube is measured in every two hours.

The results of the experiment depict that metabolism of *Saccharomyces boulardii* cells are highly dependent on the presence of Iron, Zinc and Biotin in the growth environment which can be inferred from data in Table 5. The mean values for difference in water colon heights in U-tube are 0.90 cm, 1.18 cm, 1.16 cm, 0.98 cm, 1.40 cm for controlled, iron, zinc, biotin and iron+zinc+biotin group respectively at the end of hour 10,0. As observed there is great difference between controlled group and experimental groups in which *Saccharomyces boulardii* cells were aided with external introduction of minerals and vitamins into the growth environment. As expected and stated in the hypothesis, the greatest difference in mean values with controlled group is observed in iron+zinc+biotin group. All investigated substances were introduced into this group, making it the optimum growth environment for investigated yeast species. Iron and zinc showed very similar trends and they both acted considerably on the metabolic rate of *S. boulardii*. However there is a much less difference between controlled group and biotin group which indicates that biotin is most probably the least effective substance among iron, zinc and biotin. The linear nature of the biotin line in Graph 3, also supports the hypothesis that suggested that biotin would be the least effective additive. If

there was an initial boom and very fast increase in the line of biotin group in Graph 3, then it could be suggested that biotin was extremely helpful for the growth of *S. boulardii* and it was consumed to fast and dissolved amounts were not adequate. However such great accretion is not observed in the graph and suggests that biotin is used slowly just like any other substance that is investigated. All lines show a linear nature instead of a parabolic one, further suggesting that the conditions at the end of 10 hours were still optimum. Graph 3 and Table 5 support the hypothesis and show us that if all of these minerals and vitamins are taken up together, the best effect is observed while iron and zinc show very similar properties of metabolic rates of S. Boulardii while iron is slightly more decisive in every time interval, while biotin is the least effective and sufficient substance to be investigated, it also had a considerable increasing effect on metabolism of *S.boulardii.*

Additionally, the hypothesis is advocated by the source, which explicitly state *"Some metals, like magnesium,iron, calcium, and zinc, are also required for good growth of the yeast. Concerning organic requirements, most strains of S. cerevisiae require biotin."* [10]

It can be said that there is a statistically significant mean difference between heights of water column in gas collecting apparatus due to carbondioxide release by ethanol fermantation of *S. boulardii* when *S. boulardii* were grown in media having different nutrient supplements. As P value is smaller than alpha value, the null hypothesis is rejected and

[10] Graeme M Walker, Raffaele De Nicola, Starley Anthony and Robert Learmonth.
"Yeast-metal interactions: impact on brewing and distilling fermentations" Yeast Research Group, University of Abertay.

alternative hypothesis is accepted. In short, one can state that the presence of Iron, Zinc and Biotin directly affects the metabolism rate of *S. boulardii* cells as all of them contribute to the good growth of yeast in different amounts while iron and zinc affect the specific yeast species of *S. Boulardii* at most.

The Error and Uncertainty:

In the investigation, there were some errors and uncertainities both due to the equipment used and the environment that the experiment was performed in. Although several methods were employed to minimize the error, there were some factors that posed digression. After doing the statistical analysis, one can see that both standard errors and standard deviations are small in value indicating both precision and accuracy in the investigation. Standard error is following a similar pattern for all groups indicating accuracy for all experimental groups; standard error reaches its bottom value at hour 10,0 for all experimental groups which goes down below 5% however standard deviation values are approximately 10% and precision is slightly lost in groups 3, 4 and 5 even though data preserves its general precision.

Although the standard error and standard deviation values are small, indicating accurracy and precision, and one can state that the investigation is reliable, there are some deviations as it is observed in Graph 1 as error bars.

The errors and uncertanities in the investigation were minimized by stabilizing the controlled variables such as:

- The temperature at 37,0°C via water bath,
- pH at 5,3 via buffer solution,
- equal masses of glucose is dissolved in every system,
- equal moles of ammonia is dissolved in every system,

Even though these factors were controlled, it is perceived that an experiment has error-posing components as long as it consists measurements and one can not acquire infallible results.

- Contamination by microorganisms or other non-sterile equipment could have affected the lifespan and the nature of the *S. boulardii* cells diverting their metabolism.
- Other microorganisms in water might have contributed to the evolution of gas which is eventually collected in U-tube. Sanitated water would be a better option.
- As drugs were being tested with other drugs, the method of testing should be in vivo as the acting mechanisms of drugs are usually not predictable, especially when the synergistic effects are observed.
- Gas collection is a promising yet primitive method, a digital equipment to count cells would be a much better way to indicate their metabolic rates; faster the metabolism, increased number of cell divisions thus increased number of cells.

Further investigation:

After observing the results and attaining an answer to the question **"Does the presence of Iron ions, Zinc ions and dissolved biotin in the environment, affect the metabolic rates of the Saccharomyces boulardii colonies under identical growth conditions if evolved**

carbondioxide gas is collected in an U-tube system as an indicator of metabolic rate?" a new question arises: **What are the optimum dosages for iron, zinc and biotin in order to improve the metabolism of *S. boulardii*?**

If such a question would be further investigated, then a similar method could be employed, however it would be much more intruguing to examine the effects of iron, zinc and biotin on *S. boulardii* cells inside a human body. An "in vivo" investigation can be run up on several patients diagnosed with the same kind of diarrhea that is caused by same kind of pathogen. Thus the relationship between the *S. boulardii* and the pathogen along with our investigated minerals and vitamins would be observed.

As the whole investigation struggled to come up with a synergistic substance that would boost up the metabolism of *S. Boulardii*, following up this investigation as aforementioned, would be beneficial to suggest ways to improve the potency of Reflor®.

Bibliography:

1 ,2 "Diarrhoeal disease Fact sheet N°330". World Health Organization. April 2013. Retrieved 18 June 2014.

3 "whqlibdoc.who.int" (PDF). World Health Organization.

4 Malgoire JY, Bertout S, Renaud F, Bastide JM, Mallié M (2005). "Typing of Saccharomyces cerevisiae clinical strains by using microsatellite sequence polymorphism". J. Clin. Microbiol.

5 Rajkowska, Katarzyna; et al. (April 2012). "Probiotic Activity of Saccharomyces cerevisiae var. boulardii Against Human Pathogens".Food Technology and Biotechnology 50: 230–236. Retrieved 18 January 2014.

6 Toma, Malda Maija; et al. (June 2005). "Effect of Probiotic Yeast on Genotoxicity". Food Technology and Biotechnology 43: 301–305. Retrieved 18 January 2014.

7 Soccol, Carlos Ricardo; et al. (June 2010). "The Potential of Probiotics: A Review". Food Technology and Biotechnology 48: 413–434. Retrieved 18 January 2014.

8 Li Ping Du et al., 2011, Advanced Materials Research, 343-344, 594

9 J Gen Appl Microbiol. 2008 Aug;54(4):221-7.

10 Graeme M Walker, Raffaele De Nicola, Starley Anthony and Robert Learmonth.
“Yeast-metal interactions: impact on brewing and distilling fermentations” Yeast Research Group, University of Abertay.