Федеральное государственное автономное
образовательное учреждение
высшего образования
«СИБИРСКИЙ ФЕДЕРАЛЬНЫЙ УНИВЕРСИТЕТ»
Институт фундаментальной биологии и биотехнологии
Кафедра биофизики

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МАГИСТЕРСКАЯ ДИССЕРТАЦИЯ

Определение пробиотической активности с использованием биолюминесценции АТФ

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Научный руководитель _____ PhD Р. Ранджан

Выпускник _____ Паласьос Сальседо Хоффре Сантьяго

Рецензент _____ к.б.н. М. Д. Ларионова

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Summary.

The following study establishes the ability to determine the amount and concentration of probiotics beneficial to humans in 2 different presentations commercially used in finished yogurt dairy product and tablets and determine the quality and concentration of probiotic bacteria through the use of techniques and methods of bioluminescence assay.

The two sources used for the experiment are Bio balance branded yogurt and Bion 3 branded tablets with different concentration of probiotics in its manufacture which we will determine through methods of bioluminescence and extraction of ATP obtained through results through a luminometer.

As a basic explanation of our experiment we have that ATP is an identical universal molecule for all living beings the content of ATP varies between one microorganism and another it is possible to differentiate between them through bioluminescence.

As an explanation of its functioning is an enzymatic reaction that uses an enzyme present at its origin in the fireflies Photinus Pyralis luciferase and a substrate d luciferin to obtain light from ATP and measure it with a luminometer.

The purpose of the research is to determine the quality and concentration of ATP included in amounts and concentrations are minimal with the help of our study on commercial products offered by the manufacturer and if they are met by the same through bioluminescence which can be a very interesting tool for biotech food to governments and health authorities to verify the compliance of the features offered by the manufacturer in this case products of probiotics that are beneficial for the human.

Резюме.

Нижеследующее исследование устанавливает возможность определения количества и концентрации полезных для человека пробиотиков в 2-х различных презентациях, коммерчески используемых в готовом йогурте молочном продукте и таблетках, а также определения качества и концентрации пробиотических бактерий с помощью использования методик и методов биолюминесцентного анализа.

Два источника, использованные для эксперимента, - это фирменный йогурт Bio balance и фирменные таблетки Bion 3 с различной концентрацией пробиотиков при его изготовлении, которые мы определим с помощью методов биолюминесценции и экстракции АТФ, полученных по результатам люминометра.

В качестве основного объяснения нашего эксперимента мы имеем то, что АТФ является идентичной универсальной молекулой для всех живых существ содержание АТФ варьируется между одним микроорганизмом и другим можно дифференцировать между ними с помощью биолюминесценции.

В качестве объяснения его функционирования используется ферментативная реакция, которая использует фермент, присутствующий при его происхождении в светлячках Photinus Pyralis luciferase и субстрат d luciferin для получения света из АТФ и измерения его с помощью люминометра.

Цель исследования состоит в том, чтобы определить качество и концентрацию АТФ, включенных в количествах и концентрациях минимальных с помощью нашего исследования на коммерческих продуктах, предлагаемых производителем, и если они удовлетворяются тем же путем биолюминесценции, которая может быть очень интересным инструментом для биотехнологических продуктов питания для правительств и органов здравоохранения, чтобы проверить соответствие

особенностей, предлагаемых производителем в данном случае продуктов пробиотиков, которые являются полезными для человека.

Table of contents.

1. Introduction.

Intestinal infections are a public health problem much more in developing countries such as Peru in South America or Nigeria in Africa in first World countries there are fewer risks but procuring products with high quality probiotics is an excellent way to avoid intestinal diseases by preventing them.

Systemic diarrheal syndrome is responsible for 10 million deaths annually in Africa Asia and Latin America the most common pathogenic bacteria causing diarrhea are Escherichia coli Salmonella SP Shigella SP Vibrio cholerae to which must be added viruses and parasites that with an adequate probiotic count in the human intestine can prevent intestinal diseases (Ordóñez 1985).

It is known that bacteria in the intestinal flora including probiotics constitute a protective barrier against infections and it has been suggested that the increase of these bacteria in the intestine could strengthen the same. Functional foods are intended to increase intestinal flora to strengthen the protective barrier against pathogenic bacteria some of these foods are enriched with probiotics (lactic acid bacteria similar to those that exist in the small intestine)(Eckburg 2005).

It has been suggested that functional foods could be an alternative for the Prevention of intestinal diseases and infections since the cost of them is relatively affordable for the entire population (Ordóñez 1985).

For a probiotic to be effective, it must adhere to the intestinal mucosa. To date, the adhesion of many probiotics to the intestine or its colonization has not been proven for long periods of time (Berg 1998).

1.1 Probiotics.

According to Fuller, quoted by Collins 1999, a probiotic is a food that contains living microorganisms that benefit the host by helping to maintain a microbial balance in its gut. This type of food includes fermented milk as yogurt as well as any other type of food that has been made with microorganisms. Generally, the microorganisms that are used to make these foods are all those that produce lactic acid, these include lactobacillus and Bifidobacterium (Collins 1999).

A probiotic is also considered as a non-pathogenic microorganism that when ingested continuously manages to colonize the intestine of the host quickly being able to observe beneficial changes in the health of the same (Novak 2006).

Probiotics have been subjected to countless studies in order to evaluate the possibility of using them for therapeutic purposes. They have been described as a biotherapeutic agent, which could be used to inhibit the colonization of a pathogenic microorganism in the intestinal tract (D' Souza 2002). This type of microorganisms in certain foods would protect against countless health problems, not only infectious but also autoimmune and inflammatory problems (Batista de Morais 2006).

For a microorganism to be considered as a probiotic it must meet the following characteristics:

- Being of human origin

- Not being pathogenic

- Be resistant to intestinal acids and bile

- Have the characteristics to survive in the intestinal tract

- Have the ability to influence metabolic activities (Batista de Morais 2006)

- Cause a beneficial effect on the host

- Contain a high number of viable cells

- Remain viable during storage and use, once the final product has been produced (Collins 1999).

Currently probiotics are considered as a complementary therapy to fight those pathogenic bacteria, even they are used against those microorganisms that have developed resistance to antibiotics (Berg 1998).

1.2 Benefits of probiotics.

Probiotics are attributed the following properties, among the most important are:

- Relieve symptoms of lactose intolerance

- Increase the body's defenses against intestinal infections
- Decrease serum cholesterol levels

- Assist in the process of digestion

- Stimulate gastrointestinal immunity

- Prevent colonization of the intestine by pathogens

- Strengthen the epithelial barrier

- Decrease the risk of intestinal infections caused by rotavirus (Collins 1999)

- Stimulate the production of lymphocytes and antibodies

- Increase the immune system through adjuvant effects (McCracken 1999).

The use of probiotics is widespread in the 20th century even in products of animal and human consumption more commonly dairy products such as kefir, for example.

The definition of probiotics is technically known as living micro-organisms which, when ingested in adequate quantities, produce beneficial health effects the benefits go beyond those related to basic nutrition The bacteria most commonly related to the activity probiotic are the following Lactobacillus acidophilus, L. casei, L. reuteri, L. plants, L. cheeses GG; Bifidobacterium short, B. long, B. child, B. animal; Streptococcus salivaris subespecie thermophilus, and some varieties yeast as Saccharomyces boulardii.

The mechanisms of action involved include induction at pH lower than 4, growth inhibition of pathogenic bacteria, production of lactic acid, decrease intestinal permeability, increase in the activity of lactase, competitive effect on other pathogenic bacteria, reduction in the time of elimination of rotavirus, an increase in the production of the T helper lymphocyte, and increase the secretory immunoglobulin A.

In 1965 Lilly and Stillwell were the first to quote the term probiotic1 to describe any substance or organism that contributed to maintaining intestinal balance in animals. According to these authors, it would be substances segregated by one micro-organism that stimulate the growth of another. Subsequently, in 1989, Fuller2 considered them as a living microbial food supplement that benefits the animal host with an improvement in the intestinal microbial balance. Like so many of the scientific lexicon, the probiotic word derives from the Greek word "pro-life", that is, "in favor of life", as opposed to the antibiotic term previously introduced and which means "against life". Previously, in 1908, the Russian Nobel Prize laureate Iliá Mechnikov suggested that the intake of yogurt with lactobacillos reduced the number of bacteria that produce toxins in the intestine

and contributed to the longevity of Bulgarian farmers. The most complete current definition, following Teitelbaum and Walker3, would be that of a preparation or product containing defined viable micro-organisms, in sufficient quantity to alter the microflora (by implantation or colonization) in the intestine, having beneficial effects on the host.

The criteria for microorganisms to be considered probiotics, according to Teitelbaum, may be as:

Being of human origin.

Not to be pathogenic by nature.

Be resistant to destruction by technological procedures.

Be resistant to destruction by gastric secretions and bile.

Able to adhere to the intestinal epithelium.

Be able to colonize the gastrointestinal tract, even for short periods.

Produce antimicrobial substances.

Modulate immune responses.

1.3. Functional food probiotics.

The probiotics most used in the production of fermented milk for human consumption are those belonging to the genera Lactobacillus or Bifidobacterium, these genera are used either together or each by separate.

There are studies in which it is mentioned that other non-pathogenic genera such as Escherichia, Enterococcus and Bacillus and other living organisms such as yeasts of the genus Sacharomyces have been used for the elaboration of Functional Foods (Swajewska 2006). However, it has been proven that probiotics that have a greater and better effect on human intestinal health are those of human origin (Parracho 2007).

There are two microorganisms that are not sure whether or not they should be considered as probiotics, these bacteria are Lactobacillus bulgaricus and Streptococcus thermophilus, which are and have been used over the years for the production of yogurt. It is not known whether or not they are beneficial in the intestine of the host because they do not have the necessary characteristics to be able to withstand the conditions of the human stomach, as they usually fail to reach the intestine (Swajewska 2006).

It should be borne in mind that for these foods to be effective, a minimum of colony forming units (UFCs) of probiotics is required. However, to date, no exact dose has been found in any study conducted. Some manufacturers of natural products recommend a daily dose of five billion probiotics for 5 days, this is for those probiotics that have been prescribed. To obtain therapeutic results ingesting this type of microorganism, a daily dose of between 106 and 109 UFCs per day is recommended. Although these minimum doses are.

a thorough investigation is required to determine the exact dose for the administration of this type of micro-organism (Swajewska 2006). Some authors suggest that if we want to observe a positive effect in infants to prevent diarrhea or allergies it is necessary to start supplying these microorganisms as early as possible, as their microflora begins to increase, so that they can be established in their intestine and become part of it (Batista de Morais 2006).

1.4. Probiotics in the intestine.

1.4.1. Natural origin of probiotics.

The fetal intestine is sterile, it begins to colonize from the vaginal birth where the fetus has contact with the vaginal bacteria and those of the intestinal flora of the mother. Children born by caesarean section begin colonization of their intestinal tract at the time they feed on breast milk for the first time. Those babies who are fed with breast milk have in their microflora at least 90% of Lactobacillus sp. and Bifidobacterium, while those who are fed with formulas have only 40% to 60% of these microorganisms in their gut (Batista de Morais 2006).

In addition it is necessary to mention that in the intestinal tract there is a great variety of bacteria (more than 1000 different species) that help to prevent pathogenic microorganisms colonize it. In addition in the intestine there are barriers such as acidity, peristaltic movements and elimination of

foreign microorganisms through the intestinal mucosa. These types of defense mechanisms often help to combat the colonization of pathogenic microorganisms (McCraken 1999).

1.5. The Genus Lactobacillus.

Bacteria of the genus Lactobacillus are microorganisms that can usually be found in the small intestine and vagina of humans. Some bacteria of this genus are considered beneficial because they produce vitamin K, lactase and antimicrobial substances such as acidoline, acidolfin, lactocidin and bacteriocin, which help fight and prevent infections in their hosts (Ried 2004).

Bacteria of the genus Lactobacillus are the most commonly used microorganisms as probiotics for human consumption, because they are considered safe by the fact that they have been used for several years (Berg 1998). Lactobacillus casei GG (isolated from human feces) has been reported to help a faster recovery of certain types of diarrhea, including those caused by misuse of antibiotics or Rotavirus (McCracken 1999).

Due to the great uncertainty that exists in the world on the efficacy of probiotics to colonize the gut it was decided to conduct this study in order to see if probiotics are used, above all, in enzymes for commercial production of yogurts or other functional foods are or not.

able to perform colonizations in vivo overcoming the hostile environment of the intestinal tract of a living being.

2. Overall Objective.

Demonstrate the efficacy and bacterial count of products containing probiotics through bioluminescence methods being a useful tool for quality control in the biotechnology and food industry.

3. Specific objectives.

Scientifically test the bacterial count of dairy products containing probiotics through bioluminescence methods.

To give an excellent tool to the biotechnology and food industry for quality control of this type of products.

4. Justification.

There are several studies that have postulated the benefits and multiple uses that lactic acid bacteria can have as probiotics in human health. There are already several products containing lactic acid bacteria that have been studied demonstrating their effectiveness to benefit human health. Products containing such micro-organisms are marketed in liquid form (yoghurt) or in powder form (milk), also in granules or tablets (Lin 2006). It is now necessary to study the mechanism of action by which probiotics benefit human health due to the increase of infectious diseases, autoimmune and allergies. As mentioned above, there is an expectation that these microorganisms will be an alternative in the future, including against bacteria that develop resistance to antibiotics.

The interest in finding a treatment against infectious processes has led to explore the possibility of increasing the microflora with exogenous microorganisms, such as probiotics, in order to prevent or control intestinal infectious processes. It has also been suggested the possibility of modifying the microflora in order to increase the proportion of microorganisms known to have beneficial effects. (Batista de Morais 2006).

However, so far it has not been possible to have a knowledge of the mechanism of action of probiotics in vivo, so it is not possible to perform

an accurate selection of the best strains and species that can benefit human health (Berg 1998).

There is currently much expectation about the effectiveness of probiotics in vivo. However, so far it has not been possible to verify the adhesion of these microorganisms to the human intestine or their ability to colonize it. In order to know whether or not these microorganisms have the ability to colonize the intestine as intestinal strains was made this research.

The basis of this research is the creation of a biotechnological tool for quality control of products containing probiotics and offer certified products for human and animal consumption through bioluminescence tools and publicize the benefits of this technology and its practical uses.

Exert an influence on some human metabolic activities, such as cholesterol assimilation, vitamin production, etc.

The strains most commonly used as probiotics are:

1. L. acidophilus, L. bulgaricus, L. reuterii, L. plantarum, L. casei GG (LGG).

LGG was originally selected for its resistance to gastric juices and biliary digestion, as well as for its ability to colonize the human colon. He has no plasmids, so he has stable antibiotic resistance. Produces only lactic acid (no isomer D). In its membrane it expresses adhesive factors that allow its interaction with human enterocytes. L. acidophilus can also bind to enterocytes in a calcium-independent way. Adhesion is believed to take place through an extracellular protein component. It also inhibits other anaerobic bacteria in vitro, such as Clostridium, bacteroids, Bifidobacteria, pseudomonas, Staphylococcus, Streptococcus and Enterobacteria. It also inhibits frankly pathogenic bacteria,

such as Yersinia enterocolitica, Bacillus cereus, Escherichia coli, Listeria monocytogenes and Salmonella. These effects last only as long as they are consumed; one study showed that 67% of volunteers disappeared from faeces in 7 days.

2. Bifidobacteria as Bifidobacterium breve, B. lonmgum, B. infantis, B. animalis. They constitute the most important group of sacarolytic bacteria in the large intestine, up to 25% in the adult colon, and up to 95% of the newborn with breast milk. They do not form aliphatic amines, sulphurous derivatives or nitrites, they produce vitamins, especially of Group B, as well as digestive enzymes; their metabolism produces short-chain fatty acids (AGCC), such as acetate and lactate, which decrease intestinal pH with antibacterial effects. In addition, these AGCCS are an excellent fuel for the colonocyte, and are involved in liver metabolism. 30% of ingested B. bifidum can be recovered in the HES3.

3. Streptococcus salivarius spp. Thermophilus, commonly used in the production, together with L. bulgaricus of daily consumption yoghurts. Also used L. casei, L. paracasei and L. rhamnosus.

4. The Saccharomyces boulardii fungus, with proven probiotic effects and widely used in Spain (Ultralevura®), has been shown to inhibit the growth of pathogenic bacteria in vitro and in vivo; the optimal temperature for its development is 37 ° C and has been shown to be resistant to digestion by gastric and bile juices reaching the colon free; since it is a fungus and not a bacterium, it is not affected by the concomitant use of antibiotics. After removing his administration is rapidly eliminated. Use in newborns with processes leading to malnutrition should be prudent. In 2000 we published a case of fungemia by S. cerevisiae, in two newborns, one of them treated with Saccharomyces4.

5. Another probiotic would be the kefir, who has not found godfather who studies and demonstrates its beneficial effects. Considered as a gift from Muhammad to the first converts in the Caucasus, it has only been shown to inhibit the growth of Salmonella.

However, so far it has not been possible to have a knowledge of the mechanism of action of probiotics in vivo, so it is not possible to perform.

5. Study area.

This thesis was conducted in the Laboratory of bioluminescence of the Siberian Federal University Institute of Biological and biotechnological Sciences Krasnoyarsk-Russia from November 2018 to May 2020. The products used are Kefir and yogurt containing the probiotics bioluminescent technology kits different solutions for the purpose of ATP extraction in different conceptions.

Preparation of liquid media for bacteria culture.

- 13 grams of (nutrient broth granulated) medium laboratory Himedia m002-500G.
- 1000 ml of distilled water.
- Lab crops.
- Sterilize in autoclave at 121 degrees centigrade in automatic protocol in autoclave for 1 hour and 15 minutes.

6. Materials, reagents and preparations and equipment.

- 13 grams of (nutrient broth granulated) medium laboratory Himedia m002-500G.
- 1000 ml of distilled water.
- Lab crops.
- Sterilize in autoclave at 121 degrees centigrade in automatic protocol in autoclave for 1 hour and 15 minutes.

-Sample 1 yogurt

-Sample 2 Kefir

-Buffer Tris 50 mM with BSA

-10 mkl luciferin

-10 mkl luciferaza

-5 mkl sample

-5 mkl ethanol in different concentrations.

- DMSO in different concentrations.

7. Probiotic counting.

Sample	10 -5	10 -6	10 -7
Yogurt	18	Not groth	Not groth
Kefir	70	29	22
Tablets	Liquid media	Liquid media	Liquid media

CFU Yogurt	
CFU 10 -5	7.0 x 10 7 CFU/ML
CFU 10 -6	2.9 x 10 8 CFU/ML
CFU 10 -7	2.2 x 10 10 CFU/ML
CFU Kefir	
CFU 10 -5	18x10 8 CFU (m)
CFU 10 -6	Not groth
CFU 10 -7	Not groth

8. Effect of different ATP extraction buffers in samples using bioluminescence methods.

In a buffer Tris 50 mM in different concentrations ATP indicated in our table in connection of 5 mkl of our sample and 10 mkl of luciferin and 10 mkl of luciferase we performed the verification in a luminometer of the relative unit of lumicence with the results shown in the table.

S. No.	ATP (mM)	RLU
1	10	1218923
2	8	877900
3	6	833038
4	4	491061
5	2	266911
6	1	61404
7	0.8	47480
8	0.6	35112
9	0.4	24758
10	0.2	11696
11	0.1	932

Buffer Tris 50 mM

In a Tris 50 mM buffer with BSA at different concentrations indicated in our table in connection of 5 mkl of our sample and 10 mkl of luciferin and 10 mkl of luciferase we performed the verification in a luminometer of the relative unit of lumicence with the results shown in the table.

S. No.	ATP (mM)	RLU
1	10	1052883
2	8	932499
3	6	598080
4	4	453269
5	2	223410
6	1	61100
7	0.8	46274
8	0.6	36753
9	0.4	20398
10	0.2	11106
11	0.1	354

Buffer Tris 50 mM with BSA

In ethanol at different concentrations indicated in our table in combination of 5 mkl of our sample and 10 mkl of luciferin and 10 mkl of luciferase we performed the verification in a luminometer of the relative unit of lumicence with the results shown in the table.

S. No.	Ethanol %	RLU
1	0	1823
2	10	895
3	20	855
4	50	6328
5	70	84409
6	95	84326

Ethanol.

In a combination of different reagents is a test of a buffer extraction of ATP more efficient in different concentrations indicated in our table in combination of 5 mkl of our sample and 10 mkl of luciferin and 10 mkl of luciferase, we conducted the verification in a luminometer of the unit relative luminance with the results shown in the table.

S.Nro	DMSO 100%	DMSO mkl	Ethanol 95%	Ethanol mkl	Water	RLU
1	0	0	0	0	1000	24187
2	1	10	70	736	254	15139
3	5	50	70	736	214	2126
4	1	10	50	526	464	4305

DMSO + ETHANOL + WATER.

9. Results.

9.1 Buffer Tris 50 mM

We made a medium preparation of microbiological culture of yogurt and kefir Himedia laboratory.

In the first experiment sample 1 using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 10 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 1218923 which indicates that in this concentration of ATP of our sample is high in this concentration.

In the sample 2 using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 8 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 932496 which indicates that in this concentration of ATP of our sample is optimal even in this concentration.

In the sample 3 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 6 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 598084 which indicates that in this concentration of ATP of our sample is optimal even in this concentration.

In the sample 4 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 4 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 453256 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 5 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 2 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 223459 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 6 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 1 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 61031 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 7 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 0.8 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 46174 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 8 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 0.6 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 35753 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 9 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 0.4 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 19398 which indicates that in this concentration of ATP of our sample is lower even in this concentration.

In the sample 10 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 0.2 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 11006 which indicates that in this concentration of ATP of our sample is lower even in this concentration.

In the sample 11 experiment using Tris buffer with a concentration of 50 mM we can observe the following results as higher number in a concentration 0.1 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 254 which indicates that in this concentration of ATP of our sample is very lower even in this concentration.

As a conclusion of the experiment we can say that the detection is easier and super high ATP in our sample using our Tris buffer in concentration of 50 mM in a concentration of 10 mM as higher and 0.1 mM as minimum according to our table in conclusion we say that the minimum concentration of ATP for a detection of the same acceptable would be 1 mM of ATP.

9.2 Buffer Tris 50 mM + BSA.

In the second experiment sample 1 using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 10 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 1052883 which indicates that in this concentration of ATP of our sample is high in this concentration.

In the sample 2 using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 8 mM ATP

in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 932499 which indicates that in this concentration of ATP of our sample is optimal even in this concentration.

In the sample 3 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 6 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 598080 which indicates that in this concentration of ATP of our sample is optimal even in this concentration.

In the sample 4 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 4 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 453269 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 5 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 2 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 223410 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 6 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 1 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 61100 which

indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 7 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 0.8 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 46274 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 8 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 0.6 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 36753 which indicates that in this concentration of ATP of our sample is acceptable even in this concentration.

In the sample 9 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 0.4 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 20398 which indicates that in this concentration of ATP of our sample is lower even in this concentration.

In the sample 10 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 0.2 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 11106 which indicates that in this concentration of ATP of our sample is lower even in this concentration.

In the sample 11 experiment using Tris buffer with a concentration of 50 mM + BSA we can observe the following results as higher number in a concentration 0.1 mM ATP in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 354 which indicates that in this concentration of ATP of our sample is very lower even in this concentration.

As a conclusion of the experiment we can say that the detection is easier and super high ATP in our sample using our Tris buffer in concentration of 50 mM in a concentration of 10 mM as higher and 0.1 mM as minimum according to our table in conclusion we say that the minimum concentration of ATP for a detection of the same acceptable would be 0.8 mM of ATP.

9.3 Ethanol.

In the Third experiment sample 1 using ethanol with a concentration of 96% for the first sample only water and ATP 0 we can observe the following results as higher number in a concentration 0% ethanol in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 1823 which indicates that in this concentration of ATP of our sample is lower in this concentration.

In the sample 2 using ethanol with a concentration of 96% we can observe the following results as higher number in a concentration 10 % ethanol in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 895 which indicates that in this concentration of ATP of our sample is lower even in this concentration.

In the sample 3 using ethanol with a concentration of 96% we can observe the following results as higher number in a concentration 20 % ethanol in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 855 which indicates that in this concentration of ATP of our sample is lower even in this concentration.

In the sample 4 using ethanol with a concentration of 96% we can observe the following results as higher number in a concentration 50 % ethanol in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 6328 which indicates that in this concentration of ATP of our sample is lower even in this concentration.

In the sample 5 using ethanol with a concentration of 96% we can observe the following results as higher number in a concentration 70 % ethanol in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 84409 which indicates that in this concentration of ATP of our sample is highest even in this concentration.

In the sample 6 using ethanol with a concentration of 96% we can observe the following results as higher number in a concentration 95 % ethanol in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 84326 which indicates that in this concentration of ATP of our sample is optimal even in this concentration.

As a conclusion of the experiment we can say that the detection is easier and super high ATP in our sample using our Ethanol in concentration of 96% in a concentration of 70% as higher and 10% as minimum according to our table in conclusion we say that the minimum concentration of ATP for a detection of the same acceptable would be 70% of ethanol.

9.4 DMSO + ETHANOL + WATER.

In the fourth experiment sample 1 using ethanol with a concentration of 95% + DMSO with concentration 100% + water for the first sample only water and ATP we can observe the following results as higher number in a concentration 0% ethanol 0 mkl + DMSO in concentration 100% 0 mkl + water 1000 mkl in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 24187 which indicates that in this concentration of ATP of our sample is lower in this concentration.

In the sample 2 using ethanol with a concentration of 95% + DMSO with concentration 100% + water for the second sample mixed buffer and ATP we can observe the following results as higher number in a concentration 70% ethanol 736 mkl + DMSO in concentration 1% 10 mkl + water 254 mkl in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 15139 which indicates that in this concentration of ATP of our sample is lower in this concentration.

In the sample 3 using ethanol with a concentration of 95% + DMSO with concentration 100% + water for the third sample mixed buffer and ATP we can observe the following results as higher number in a concentration 70% ethanol 736 mkl + DMSO in concentration 5% 50 mkl + water 214 mkl in combination of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 2126 which indicates that in this concentration of ATP of our sample is very lower in this concentration.

In the sample 4 using ethanol with a concentration of 95% + DMSO with concentration 100% + water for the fourth sample mixed buffer and ATP we can observe the following results as higher number in a concentration 50% ethanol 526 mkl + DMSO in concentration 1% 10 mkl + water 464 mkl in combination

of luciferin and luciferase in volumes of 10 mkl and 5 mkl sample we obtain the highest result an RLU of 4305 which indicates that in this concentration of ATP of our sample is very lower in this concentration.

As a conclusion of the experiment we can say that the detection is easier and super high ATP in our sample using our Ethanol in concentration of 95% + DMSO with concentration 100% + water number in a concentration 0% ethanol 0 mkl + DMSO in concentration 100% 0 mkl + water 1000 mkl as maximum according to our table in conclusion we say that the minimum concentration of ATP for a detection of the same acceptable would be 70% ethanol + DMSO in concentration 5% 50 mkl + water 214 mkl.

As a conclusion we can say that this mixed buffer is not a suitable ATP extraction buffer.

10. Discussion.

As a discussion I can argue that experimenting with different types of ATP extraction buffers is an appropriate and successful option to observe results for detection of microorganisms at very low levels in this case in our research are probiotics through the use of methods and technology of bioluminescence specified based on luciferin and luciferase.

We can say that the technology of bioluminescence is an excellent tool for modern biotechnology in our case the design of a tool for quality control and detection of microorganisms in the field of biotechnology and food in this way we will put more challenges to producers to develop food products of exceptional quality and also demonstrate on a scientific basis the food products of lower quality.

11. Conclusions.

The protocol used in this thesis has proved to be a valid scheme to evaluate the capacity of different ATP extraction buffers used independently and combined and determine the most optimal for their purpose ATP extraction of microorganisms.

It was proved that the use of bioluminescence technology in combination with a good ATP extraction buffer is a viable and useful tool for food biotechnology to perform an adequate quality control of different food products based on microorganisms such as kefir and yogurt used in this thesis for the detection of probiotics even in very low concentrations.

12. Recommendations.

In my opinion it is a very useful biotechnological tool in the field of Food Biotechnology and we should continue to test different combinations of exists buffers and test new ones to achieve a more accurate detection in extremely small quantities.

Making bioluminescence technology more relevant by having a very promising future in the short term.

13. Limits.

The possible limitations in this technology and in this serious thesis that has a limit of detection of microorganisms in concentrations of them very low.

14. Literature.

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15. Figures.

Fig 1. Luminometer

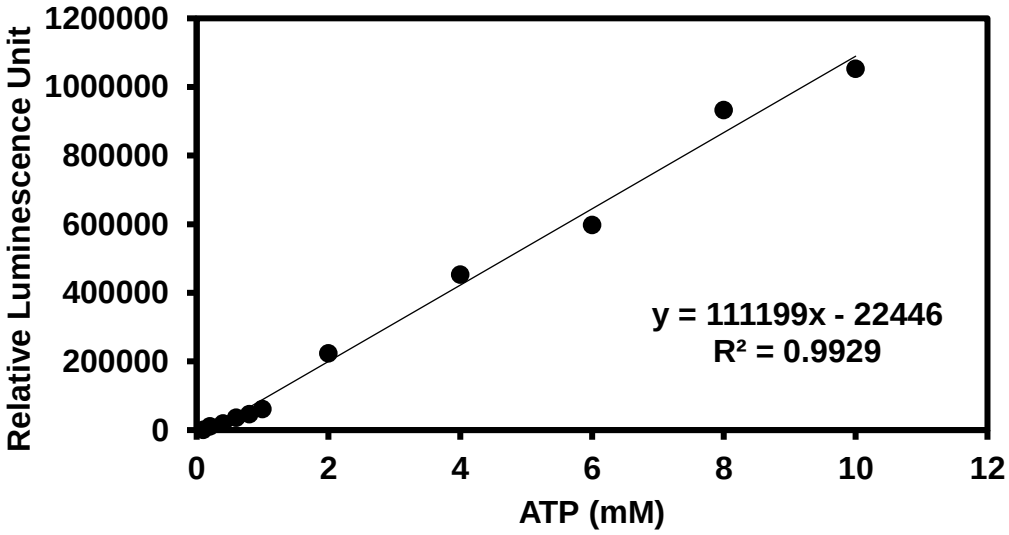
Fig 2. Balance reagents and pipettes

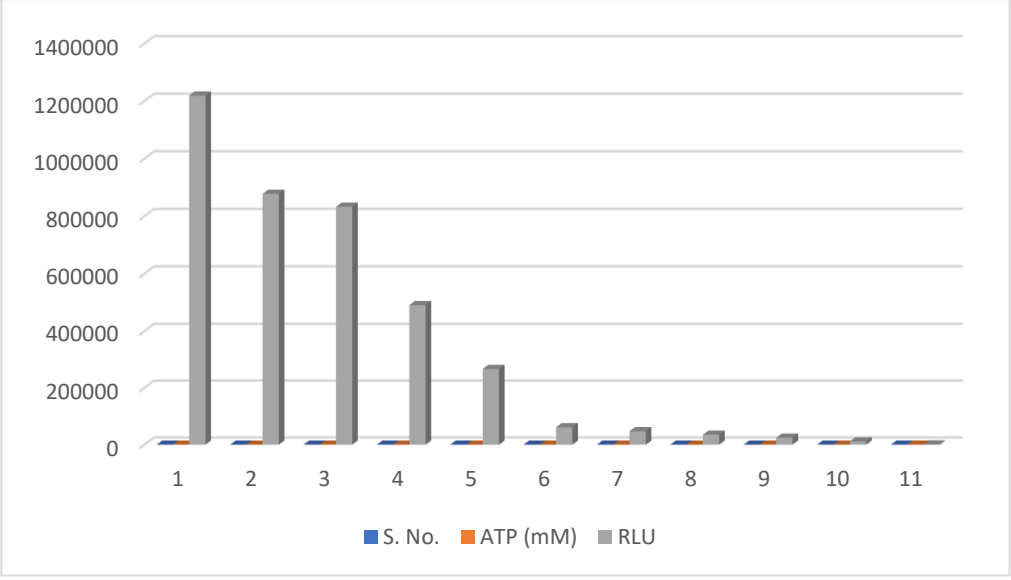
Fig 3 Type 2 biosecurity laminar box

Fig 4. Autoclave.

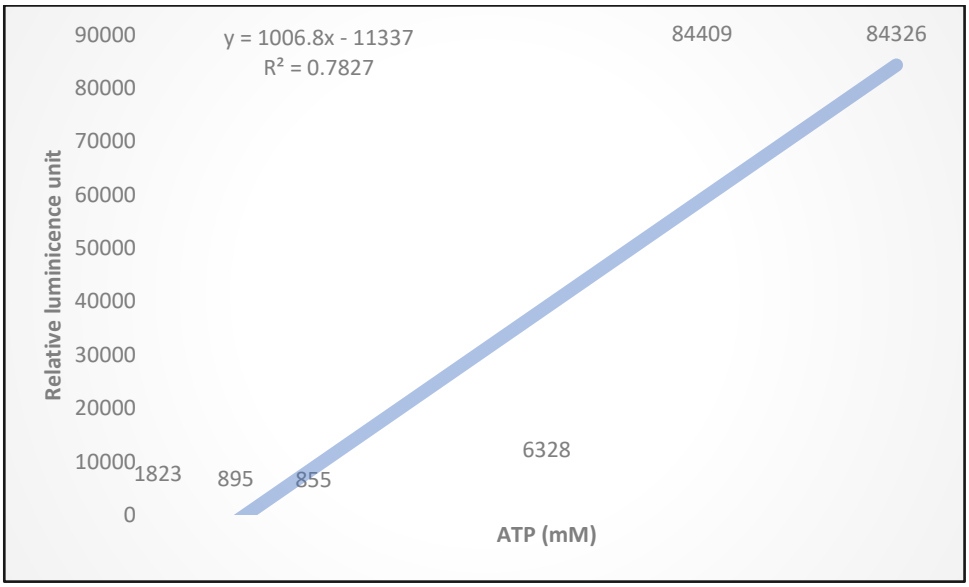
16. Graphics.

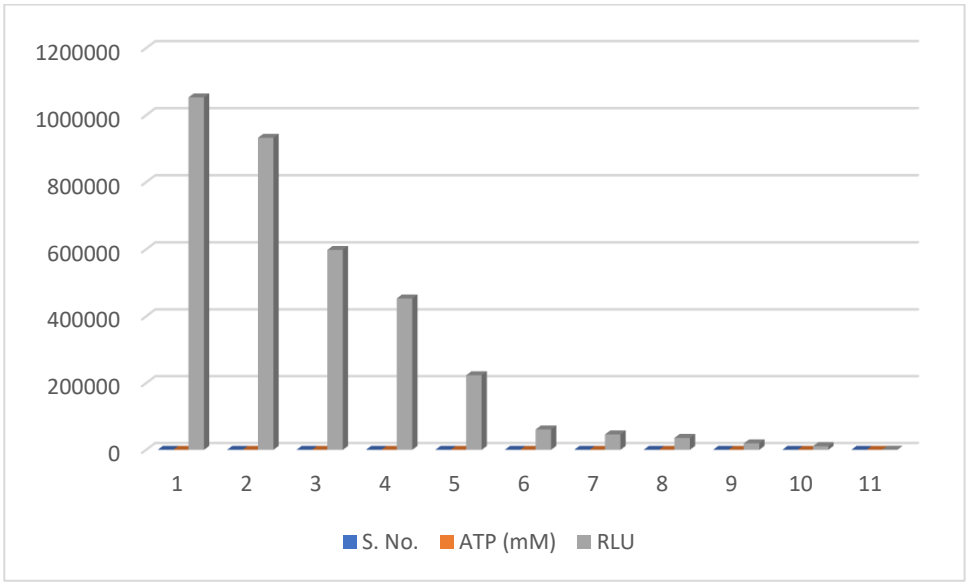
7.1 Buffer Tris 50 mM



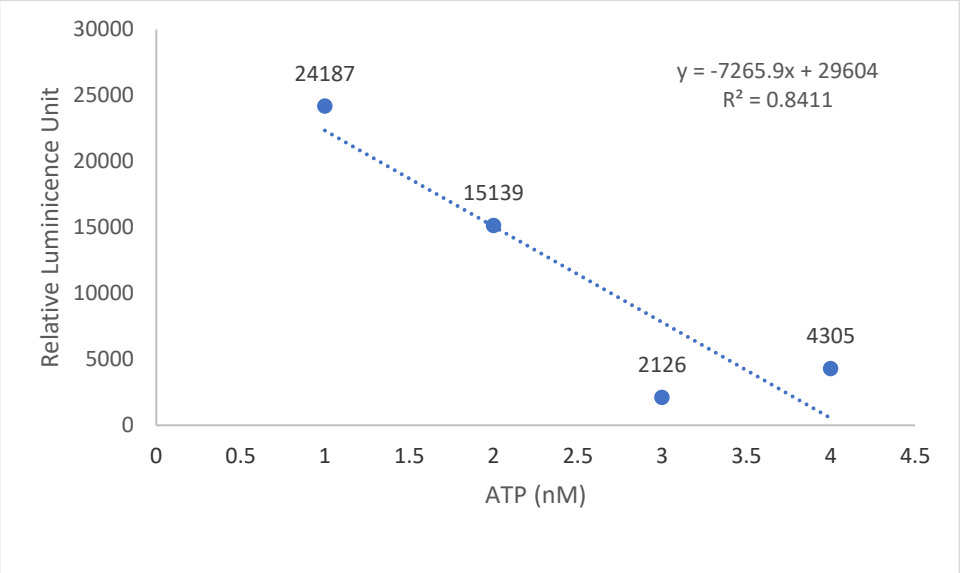


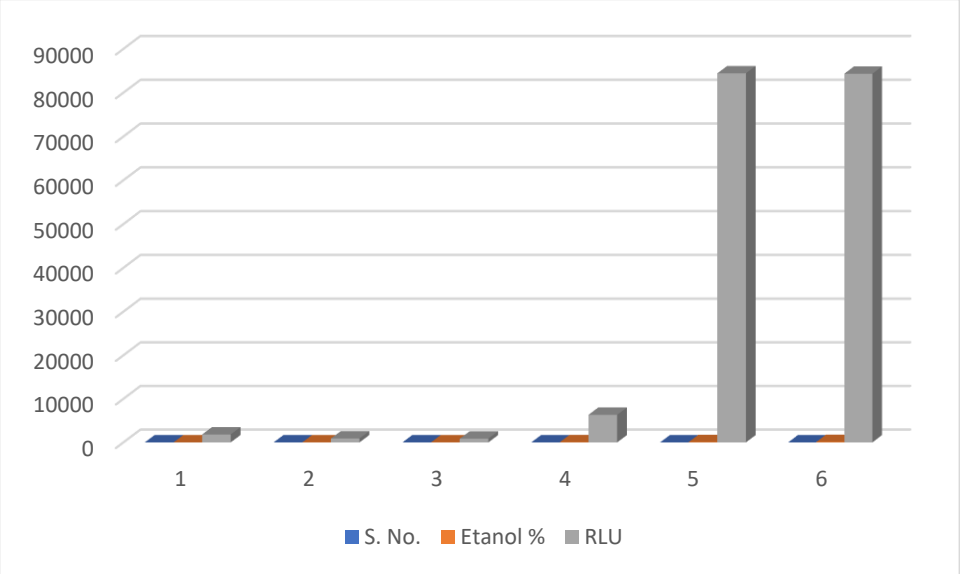
7.2 Buffer Tris 50 mM + BSA.



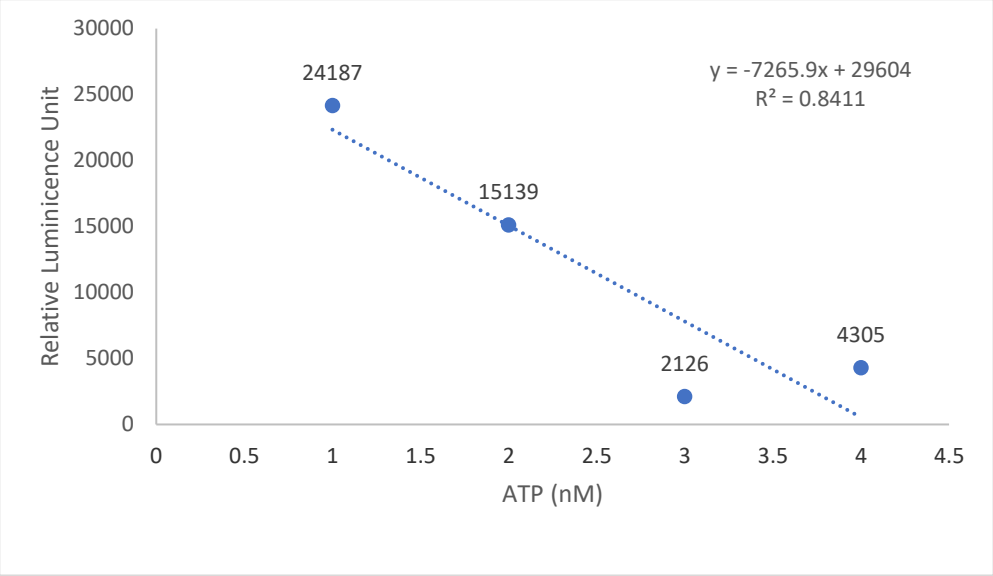


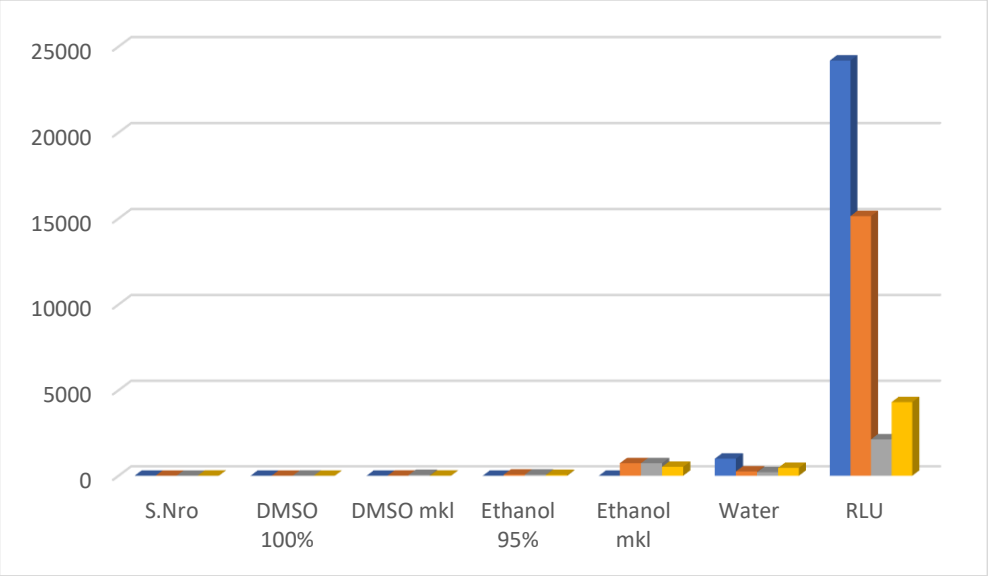
7.3 Ethanol.





7.4 DMSO + ETHANOL + WATER.


30000
25000
20000
15000
10000
5000
0
Relative Luminicence Unit
24187
15139
2126
4305
y = -7265.9x + 29604
R² = 0.8411
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
ATP (nM)


25000
20000
15000
10000
5000
0
S.Nro
DMSO 100%
DMSO mkl
Ethanol 95%
Ethanol mkl
Water
RLU

The Federal State Autonomous
Educational Institution of Higher Education
"Siberian Federal University"
Institute of Fundamental Biology and Biotechnology
Department of Biophysics

CONFIRM

Head of Department

/V.A. Kratasyuk /

«24» июня 2020

MASTER'S THESIS

Determination of probiotic efficiency using ATP bioluminescence

06.04.01 Biology
06.04.01.10 Biological Engineering

Scientific advisor 22.06.2020 Dr R. Ranjan

Master Student 22.06.2020 S. K. Palasos Salsedo

Reviewer 23.06.2020 Dr M.D. Larionova

Krasnoyarsk 2020

Федеральное государственное автономное
образовательное учреждение
высшего образования
«СИБИРСКИЙ ФЕДЕРАЛЬНЫЙ УНИВЕРСИТЕТ»
Институт фундаментальной биологии и биотехнологии
Кафедра биофизики

УТВЕРЖДАЮ

Заведующий кафедрой

/В.А. Кратасюк/

«24» июня 2020 г.

МАГИСТЕРСКАЯ ДИССЕРТАЦИЯ

Определение пробиотической активности с использованием биолюминесценции АТФ

06.04.01 Биология
06.04.01.10 Биологическая инженерия

Научный руководитель 22.06.2020 PhD Р. Ранджан

Выпускник 22.06.2020 Паласьос Сальседо Хоффре Сантьяго

Рецензент 23.06.2020 к.б.н. М. Д. Ларионова

Красноярск 2020