THE ANTIBACTERIAL EFFECT OF FIVE KIND OF SOLID SOAPS (DAISY, ROSE, BAY, CARNATION, OLIVE OIL) ON THE ACNE CAUSING BACTERIA: *Propionibacterium acnes*

Session:	May 2013
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IB Subject of Essay:	Biology
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Word Count:	3597

ABSTRACT

This experiment evaluates the antibacterial effect of solid soaps on the acne causing bacteria which is called *Propionibacterium acnes*. Acne is a skin disease which occurs because of this type of bacteria and a soap is a chemical matter which is used for cleaning. The research question of this investigation is : "**Does the different kind of solid soaps given to the *Propionibacterium acnes*, including daisy, rose, bay, carnation and olive oil, affect the bacteria which are in identical conditions and have an average length of 1.5 nanometers by measuring the length after adding the soap solutions with the help of a compound microscope at 34ºC and 1067 hPa pressure?"**

In the experiment, I got the sample of bacteria to use from a person's skin and made it reproduce in a blood agar base which sets an optimum condition for bacteria to reproduce. I prepared solutions including 5 grams of soap and 50 mL water for every kind of soap. I put the solutions in the bacterial cell and measured the change in the length of it. This method is similar to Kirby- Bauer method but it differs in some ways. I also prepared a control group to be sure the response of bacterial cell to solution is caused because of the soap. I used ANOVA-Single Factor to analyze my quantitative data.

I concluded that solid soap effect the size of the bacteria used in a positive way according to a teenager in other words the solution including the soap caused the bacteria become smaller. In addition, the most effective soap is found to be bay soap while the least effective one is carnation soap.

(275 words)

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INTRODUCTION & BACKGROUND INFORMATION

I am 17 years old which is an age of puberty. In this stage of human life, so many changes occur in our body, both physical and hormonal. In hormonal way, body becomes ready to fertilization and in girls ovaries, in boys testes starts to develop. In physical way, the height and weight accelerates, voice becomes squeaky, and hair starts to grow in –almost - every part of the body. For teenagers, the greatest problem of puberty is acnes which form in the face and do not disappear in a short time period. In addition, it can leave its mark which can last until the end of our lives.

Acne is described as a skin disease which occurs in face, back, shoulders and chest of a human. It is mostly seen between the ages 14 and 20, which are the ages of puberty. The typical symptom of acne is black spots. [1]

In medical, the formation of acne starts with a plugged canal of an oil gland. The head of this canal becomes tough and turns into black. Sometimes, gland keeps secreting oil although the canal is plugged and a cyst is formed which is full of oil. Those black spots are called "comedon". After the comedon formed, the bacteria, *Propionibacterium acnes*, settles in here and contributes the formation of acne. [2]

The most important matter of acne to occur and the subunit of it is the bacteria *Propionibacterium acnes*. It is the most common gram-positive, non-spore forming, anaerobic rod encountered in clinical specimens. *P. acnes* typically grows as an obligate anaerobe, however, some strains are aero tolerant, but still show better growth as an anaerobe. It has the

[1] http://www.medicinenet.com/acne/article.htm

[2] http://www.medicinenet.com/acne/article.htm

ability to produce propionic acid, which is used for making foods' surface resistant to mildew, as its name suggests. It also has the ability to produce catalase along with indole, nitrate, or both indole and nitrate. [3]

Propionibacterium acnes is normal residents of the skin and is generally harmless. But, when pores become engorged with excess oil and dead skin cells, it creates an anaerobic environment where P. acnes can thrive. P. acnes triggers inflammation within the pore, creating a papule, pimple, or cyst. Reducing the P. acnes population is an important step in controlling acne.[4]

Its kingdom is Bacteria, phylum is *Actinobacteria*, order is *Actinomycetales*, family is *Propionibacteriaceae.*

Most of my friends tried to make the acnes disappear lots of times. They tried too many ways such as; salving cream or making the acne burn with some kind of laser light. But they have never tried washing their skin with a soap which includes organic matters. The reason why I am investigating this subject is to show people a way to make their acnes disappear or heal in a short time period.

Soap is the salt which is produced from the reaction of an acid and a base, in chemistry. It can be in the shape of a mould or liquid which have matters giving reaction with water. It used as surfactants for washing, cleaning and bathing and obtained by treating vegetable or animal oils with a strongly alkaline solution. [5]

In history, the first signs of soap are found in 2800 B.C. from the inscriptions from Babylon. According to the myth, women were washing the clothes near the river below the

[3] http://web.mst.edu/~microbio/bio221_1998/p_acnes.html
[4] http://acne.about.com/od/acneinformationglossary/g/pacnes.htm
[5] http://en.wikipedia.org/wiki/Soap

hill where people sacrifice animals. By the rain, the animal oils and wastes came down to the river and combined with the water. Later, women saw that clothes became cleaner by using this substance. And most importantly, the first formula of soap was written on an inscription in 2200 B.C. again in Babylon.[6]

There are lots of kinds of soap found in a market. In this investigation, I will be working with solid soaps which means they include matters all from the nature. I will use the soaps including daisy, rose, bay, carnation and finally olive oil. The basic qualities of these kinds of soaps are:

1) Daisy soap: makes the skin more rigid and bright, helps the healing process of allergic situations, and makes the itches caused by eczema disappear.

2) Rose soap: makes the birthmarks disappear, helps the cleaning of dead cells on the skin and moisturizes the skin.

3) Bay soap: prevents the hair to be shed, opens the pores and makes the skin relaxed.

4) Carnation soap: regulates the metabolism, has an antiseptic effect and prevents skins to be wrinkled.

5) Olive oil soap: increases the resistance of skin in case of any skin disease, makes the skin look bright and clean. [7]

Throughout this essay, I will be researching and making an experiment about my topic, the antibacterial effect of organic solid soaps on the bacteria causing the formation of acne in puberty.

[6] http://www.goodscentscandles.us/soaphistory.php
[7] http://www.elruhasabun.com/

My research question is: "**Does the different kind of solid soaps given to the *Propionibacterium acnes*, including daisy, rose, bay, carnation and olive oil, affect the bacteria which are in identical conditions and have an average length of 1.5 nanometers by measuring the length after adding the soap solutions with the help of a compound microscope at 34ºC and 1067 hPa pressure?**"

HYPOTHESIS

Acne is caused by the bacteria called *Propionibacterium acnes*. The formation of acne starts with secretion of oil from the gland and as the oil keeps stored, black spots occur. When this bacteria comes and settles in that region, the acne is produced. To make an accurate hypothesis the literal mean of "antibacterial" must be known. An antibacterial is an agent that inhibits bacterial growth or kills bacteria.[8]

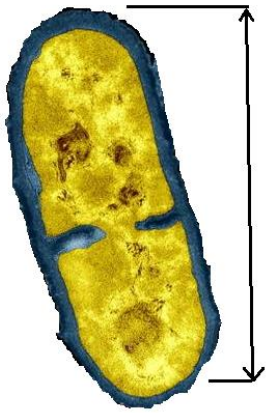
As explained in introduction part, I will be using 5 different kinds of solid soaps. In this investigation the antibacterial effect of these soaps on the length of the bacteria – which has approximately 1.5 nanometers length [9] - will be compared by using a compound microscope. (See *Appendix 1*)

It is hypothesized that the solutions given to the bacteria in the same conditions will make the bacteria smaller than it was before starting the experiment. Because the section of the bacteria where I will drop the solution on, will become like an unliving cell and all of the metabolic activities taking place in will stop. It will affect the length of it. So, it will become smaller in terms of μm.[10]

[8] http://en.wikipedia.org/wiki/Antibacterial
[9] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735618/
[10] http://www.cnklp.com/sldd/walk_upfile/200773111213962.pdf

METHOD DEVELOPMENT AND PLANNING

In this investigation, the antibacterial effect of 5 different solid soaps on the length of bacteria used will be compared. This comparison will be done by analyzing the quantitative data collected - by performing Anova-Single Factor - from the experiment. Quantitative data will be recorded by measuring the length of the cell of the bacteria, which causes acne on the skin, *Propionibacterium acnes* before dropping the chemicals – soap concentrations- and after they are dropped. I expect to see a circle, or a zone, occurred by the existence of a drop of the soap concentration. Of course, because of the diffusion, the matter will diffuse all over the cell and make it smaller.



Length of the bacteria in terms of nanometers.

Figure 1: The transmission electron micrograph of *P. acnes*.

According to the Kirby-Bauer antibiotic testing method[11] , the circle - formed because of the chemical matter- is the area any cellular activities take place anymore; in other words the matter dropped makes the cellular activities stop. As the matter diffuses into the cell, it will affect the entire cell and finally cell will be destroyed.

[11] http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol

To sum up, by the method which I will use to compare the antibacterial effect of 5 different kinds of solid soaps, I will measure the length of the cell. If the matter is effective, then the length of the cell will become smaller or if the matter is not effective, then the length of the cell will remain same in the same time interval.

So; the independent variable is the ingredient of soap, the dependent variable is length of the bacteria cell observed and photographed by the compound microscope, then transferred into a computer and measured, and controlled variables are type of the bacteria, amount of soap solution used and time interval.

This method is likely to the Kirby-Bauer method which supports measuring the diameter of the circle. Mine and Kirby-Bauer are both provide quantitative data to be analyzed and both measuring the length. I have never seen a kind of method which I will use before but in my opinion it will work for my experiment, because the diffusion of the chemical matter in the cell will make the cell activity stop and after a while, because of the lack of protein or other living causing matters, the cell will shrink so the length will become smaller.

I selected my soaps as including natural ingredients because I do not want the presence of any chemical or dangerous substances in the material. I preferred using the soaps including daisy, rose, bay, carnation and olive oil. I selected the same brand for the soaps: El Ruha ®. In the experiment, before I put the soap into the bacteria, I will mix the soap with 50 mL water so that I can count number of drops. In that way, the amount of solution put in every bacteria will be equal and I can control that.

Propionibacterium acnes will be used in the experiment with the purpose of creating a general conclusion for every young people at the age of puberty and having acnes.

The experiment will be performed in the Hacettepe University – Pharmacology Faculty. Professor Doctor Filiz YALÇIN will be helping me during the experiment and she will find the equipment needed for my experiment.

The sample of this bacteria will be taken from a person's skin and will be reproduced in a system which is lack of oxygen gas because the bacteria is anaerobe[12] which means it does its respiration without oxygen gas. After taking the sample, it will immediately be placed into a tube which will not have oxygen in it and it must be got into the laboratory without wasting any time.

In laboratory, there will be a system called "blood agar base" (See *Appendix 2*) which will be set before. It provides a place for every kind of bacteria to produce faster than it normally does. For anaerobe bacteria, this system is set with a vacuumed place. This vacuumed place has 85% N, 10% H and 5% CO_2 gases in it. In addition, it is at ideal temperature for the bacteria. For *Propionibacterium acnes* the ideal temperature is the temperature of skin, 34ºC[13] . After placing the bacteria into the system, I will wait for 3 days to obtain a great amount of bacteria to work on.

At the end of the 3rd day, I will have my bacteria in the system and I will observe them with a special microscope called "compound microscope"(See *Appendix 1*).

As I drop the concentration, prepared with each soap and water in the ratio of 20 grams of soap and 50 mL water of soaps, I will wait 10 minutes for diffusion. (10 minutes will be enough because I made a try before.) And I will record the data (length of the

[12] Dali, P.; Giugliano, E. R.; Vellozzi, E. M.; Smith, M. A. (2001). "Susceptibilities of Propionibacterium acnes Ophthalmic Isolates to Moxifloxacin". *Antimicrobial Agents and Chemotherapy* **45** (10): 2969–70. doi:10.1128/AAC.45.10.2969-2970.2001. PMC 90767. PMID 11583007

[13] http://www.healthyheating.com/Definitions/facts_about_skin.htm

bacteria). I will be measured the real length of the bacteria before I put the solution. I will compare the ratios of change for different soaps.

But there is a question on my mind:"How can I be sure that the change in length is caused because of the soap that I put in?" To examine this issue, I decided to make a control group which I will not put soap but just water. If the size of the bacteria in this group does not change, I will become sure that it is the soap which has the antibacterial effect on the bacteria.

As I mentioned above, I will use 5 different soaps. For 1 type of soap, I will perform 5 trials. So, when the experiment is finished, I will have 25 values in total. These values will be analyzed by calculating their mean, median, range, variance, standard deviation, standard error, t- value, %95 Cl (SEXT (0,05 DT) and %95 Cl (EXCEL) and also performing ANOVA- Single Factor.

At the end of my investigation, I expect to see a smaller cell which I dropped the concentration of solid antibacterial soap in. Because the cellular activity will stop and it will make the bacteria cell shrink. This causes a change in the length.

MATERIAL LIST

- 1 mold of El Ruha® Daisy Soap
- 1 mold of El Ruha® Rose Soap
- 1 mold of El Ruha® Bay Soap
- 1 mold of El Ruha® Carnation Soap
- 1 mold of El Ruha® Olive oil Soap
- Sample of *Propionibacterium acnes* taken from a human's skin.
- Blood Agar Base
- Compound Microscope x1
- Petri Dish x5
- 500 mL distilled water
- Graduated Cylinder (50 mL) x5
- Chronometer
- Thermometer

DATA COLLECTION AND PROCESSING

Types of solid soaps used	Type of bacteria used	Trials	Length of the bacteria in the beginning (± 0.1 µm)	Length of the bacteria at the end (± 0.1 µm)	Change in length of bacteria (± 0.1 µm)	Time passed during the diffusion (± 0.1 min.)	Temperature (± 0.1 ºC)	Pressure (± 0.1 hPa.)
Daisy	*P. acnes*	1	1.5	1.1	0.4	10.0	34	1067.0
		2		0.8	0.7			
		3		0.9	0.6			
		4		1.0	0.5			
		5		1.1	0.4			
Rose	*P. acnes*	1	1.5	1.3	0.2	10.0	34	1067.0
		2		1.1	0.4			
		3		1.2	0.3			
		4		1.2	0.3			
		5		1.1	0.4			
Bay	*P. acnes*	1	1.5	0.6	0.9	10.0	34	1067.0
		2		0.6	0.9			
		3		0.5	1.0			
		4		0.6	0.9			
		5		0.7	0.8			
Carnation	*P. acnes*	1	1.5	1.3	0.2	10.0	34	1067.0
		2		1.4	0.1			
		3		1.3	0.2			
		4		1.3	0.2			
		5		1.2	0.3			
Olive Oil	*P. acnes*	1	1.5	0.9	0.6	10.0	34	1067.0
		2		0.7	0.8			
		3		0.8	0.7			
		4		0.7	0.8			
		5		0.9	0.6			

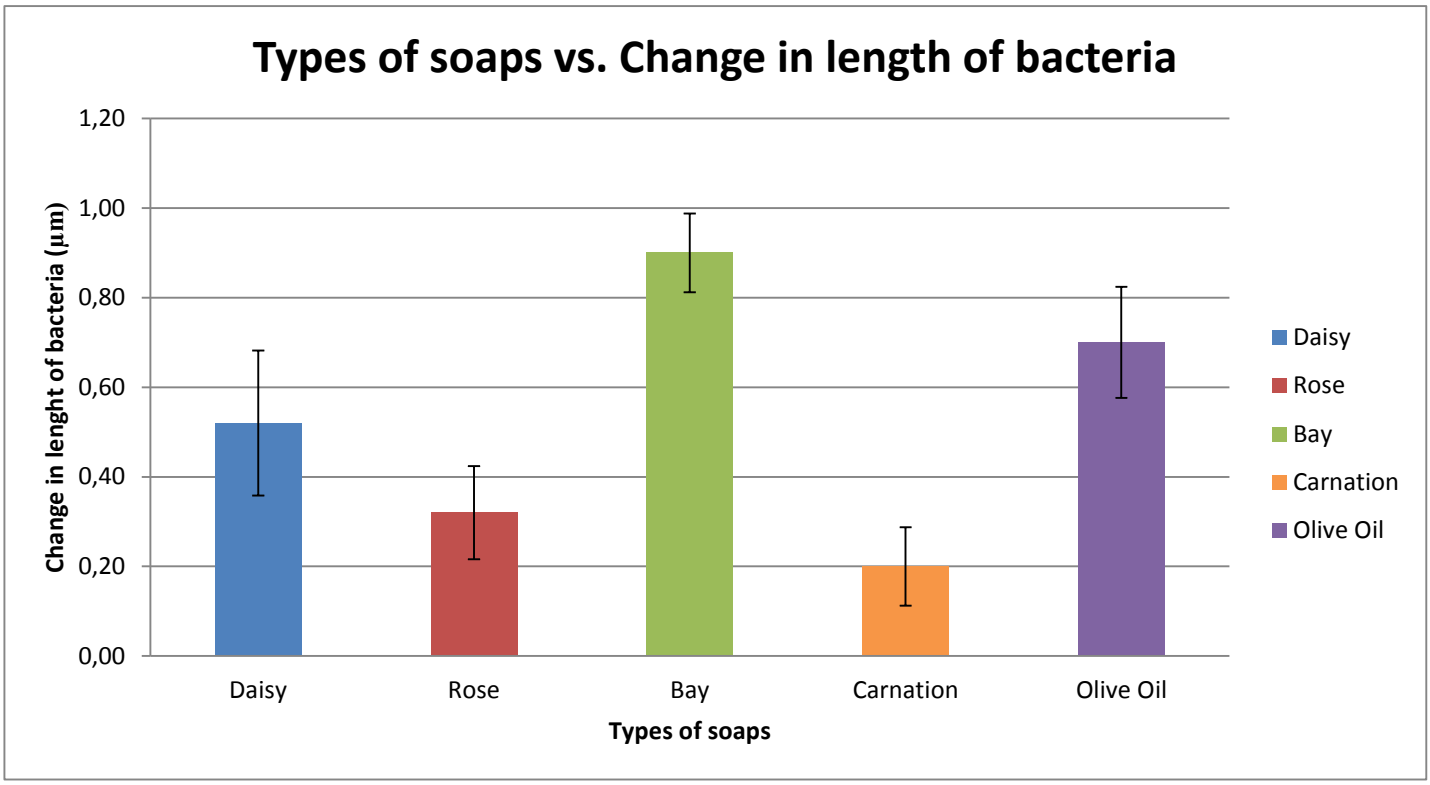
Table 1: This table shows the types of soaps used, the length of the bacteria before and after putting the solution and change in the length of bacteria with the controlled variables temperature and pressure.

	Daisy	Rose	Bay	Carnation	Olive Oil
MEAN	0,52	0,32	0,90	0,20	0,70
MEDIAN	0,5	0,3	0,9	0,2	0,7
RANGE	0,3	0,2	0,2	0,2	0,2
VARIANCE	0,017	0,007	0,005	0,005	0,01
SD	0,130384	0,083666	0,070711	0,070711	0,1
SE	0,05831	0,037417	0,031623	0,031623	0,044721
T	2,776445	2,776445	2,776445	2,776445	2,776445
%95 Cl (SEXT (0,05 DT)	0,161893	0,103885	0,087799	0,087799	0,124166
%95 Cl (EXCEL)	0,114285	0,073335	0,06198	0,06198	0,087652

Table 2: This table shows the mean, median, range, variance, standard deviation, standard error and t values of the change in length of bacteria values.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Daisy	5	2,6	0,52	0,017		
Rose	5	1,6	0,32	0,007		
Bay	5	4,5	0,9	0,005		
Carnation	5	1	0,2	0,005		
Olive Oil	5	3,5	0,7	0,01		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1,5944	4	0,3986	45,29545	9,43E-10	2,866081
Within Groups	0,176	20	0,0088			
Total	1,7704	24				

Table 3: The Anova: Single Factor Table of data collected.



Graph 1: The column chart of mean values of change in length of the bacteria versus the kind of soaps using 95 CI as error bars.

CONCLUSION AND EVALUATION

In this experiment, the antibacterial effect of 5 different solid soaps is investigated on the bacteria which cause the acne, *Propionibacterium acnes.* The sample of bacteria is taken from real acne, from a person and in laboratory conditions an appropriate agar base is prepared. In that agar base, the sample bacteria began to reproduce and at the end of 3 days I had an amount of bacteria which was enough for my experiment. Because of the bacteria being an anaerobe, the agar base was lack of oxygen but mostly nitrogen and hydrogen gases. The optimum temperature was also set, which is 34ºC for this type of bacteria.

5 different kinds of soaps were used to test their antibacterial effect which were including some natural ingredients: daisy, rose, bay, carnation and olive oil. The same weight of piece of soap is taken from that soaps, 20 g. And this piece is mixed with same amount of water – 50 mL - in order to put the soap in the bacteria easily and fix the amount of chemical put in each bacteria cell. A droplet was used to add soaps to cells.

To test the soaps' antibacterial effect a method which is so close to the Kirby-Bauer method is used. Kirby-Bauer method requires the measurement of the circle formed because of the chemical put in the bacteria cell but I have chosen to measure the length of the whole bacterial cell. My method differs from Kirby-Bauer because of this reason. But putting of the chemical (like soap) or type of measuring size is similar.

Before starting the experiment, the length of the bacteria cells was measured by observing them with a special microscope called compound microscope. This tool is specialized with its feature of giving us the chance to see any single small particle, like the bacteria used, in a detailed and larger way. The average length is found as 1.5 (± 0.1) μm. After dropping the soap solution, the new length of the bacteria was recorded after 10 minutes

passed, which was counted by a chronometer. At the end of the experiment, there was a change in lengths of bacteria which were put the soap solution in. The new length of the bacteria was compared with 1.5 μm and the difference was found. The statistical analysis was done on these difference values.

The hypothesis made before was saying that the lengths of the cells will become smaller and the result of the experiment proves this.

The statistical analysis (ANOVA) was done (see *Table 3*) and as a result a P-value was found. The P-value is 9.43E-10 (9.34×10^{-10}). This value calculated is less than the alpha value which is 0.05. P-value being less than alpha value proves that the hypothesis made before is true and needs to be accepted with 95% confidence. So it can be said that solid soaps effect the size of the bacteria and directly the size of the acne. The truth of the hypothesis can also be proved by checking the mean values of the change in the length of the cell. (See *Table 2*). When the soap containing bay put into the bacterial cell, the mean of the difference in terms of length is 0.90 μm which is the highest mean value of them all. But putting the soap solution containing carnation in the same conditions with others made the mean value 0.20 μm which is the least value. Based on these quantitative data, it is clear that using soap effects the size of the bacteria. But there is another result, as well. The type of the soap also effects the difference in length of the cell. As it is said above, the solution containing bay caused the bacteria smallest compared to other types of soaps used in this investigation while the one with carnation caused the least change. It can be said that using soap including bay will make the acne become smaller.

When the standard error values are checked, it can be seen that they are very low. The highest one is 0.05831 which belongs to the daisy soap. This value equals to 5% and this is a low value, as well. Less error means that the result I got is accurate.

To determine to most homogenous distribution, there must be the least standard deviation and error because if the standard deviation increases, the errors made increases, as well. When these values are checked (See *Table 2*), there are not any value which is too high; the highest one is 0.130384 which equals to 13 %. But the soaps containing bay and carnation have the least and same values for both standard deviation and error. This means that the most homogeneous data collected are belong to these 2 kinds of soap. In addition, low standard deviation and error shows that our results are precise. According to the explanation above, it can be said that the results of this experiment is precise, too.

Another way to prove the hypothesis made or to check the result of the experiment made, is to find a literal information which was found as a result of an experiment made before and made in the same direction with this investigation. According to an experiment done in Department of Dermatology, Ulsan University Hospital, Ulsan, Korea[14] , the number of lesions occurred because of acne is reduced by using 2 different types of cleaners.

Even though, they cleared at different amounts, the result of the investigation is able to prove the result of my investigation.

Although some variables tried to keep constant, there were some error sources that can effect the result of the experiment.

- Firstly, after the reproduction of the bacteria cells in blood agar base, the cells were not in the same length. The cells which have closer length values to the mean value

[14]http://acne.about.com/gi/o.htm?zi=1/XJ&zTi=1&sdn=acne&cdn=health&tm=149&gps=465_12_1280_621&f=00&su=p284.13.342.ip_&tt=12&bt=1&bts=1&zu=http%3A//www.ncbi.nlm.nih.gov/pubmed/20394494

are used. While making calculations the mean value is used. This can effect the result directly.

- The sample of the bacteria is taken from a person and transported to the laboratory in a tube which was lack of gas. But explained in Method part, this bacteria need some other gases like nitrogen. This condition could not set during the transportation.

To eliminate these error sources and improve this experiment, the followings can be done:

- If it is not possible to arrange bacterial cells having same length value, then the bacteria cells having the same or so close length values can be separated and used. By this way the difference in length can be measured more accurately.

- The person who is the donor of the bacteria sample, can come to the laboratory and the sample can be taken there. So the sample of the bacteria will not be affected this much from the lack of the gases.

To summarize, the ones who are at their age of puberty and having the acne problem, can use the solid soaps without any stress because this investigation has proved that the soaps make the acne causing bacteria become smaller and this will cause a smalling in the size of acne. I am advising the usage of the BAY soap.

I have made this investigation and found out that solid soaps effect the size of the acne. If someone wants to make an investigation about that subject, his/her research question can be: “Which type of soap causes a better result in the size of acne?” and can use liquid soaps or antibacterial soaps. By this way, maybe another cure for acnes can be found and teenagers will not be stressed about their acnes on their faces anymore.

APPENDIX

Appendix 1:

Figure 2: The compound microscope which I will use to observe the change in the bacteria.

The common light microscope used in the laboratory is called a compound microscope because it contains two types of lenses that function to magnify an object. The lens closest to the eye is called the ocular, while the lens closest to the object is called the objective**.** Most microscopes have on their base an apparatus called a condenser, which condenses light rays to a strong beam. A diaphragm located on the condenser controls the amount of light coming through it. [15]

[15] http://www.cliffsnotes.com/study_guide/Types-of-Microscopes.topicArticleId-8524,articleId-8412.html

Appendix 2:

Figure 3: The blood agar base with reproducing microorganisms.

Blood agar plate (BAP) contains mammalian blood (usually sheep or horse), typically at a concentration of 5–10%. BAP are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity. β-hemolytic activity will show lysis and complete digestion of red blood cell contents surrounding colony. Examples include *Streptococcus haemolyticus*. α-hemolysis will only partially lyse (the cells are either lysed or not- it is the digestion that may be incomplete) the hemoglobin and will appear green. An example of this would be *Streptococcus viridans*. γ-hemolysis (or *non-hemolytic*) is the term referring to a lack of hemolytic activity. Contains meat extract, tryptone, sodium chloride, and agar.[16]

[16] Fisher, Bruce; Harvey, Richard P.; Champe, Pamela C.. *Lippincott's Illustrated Reviews: Microbiology (Lippincott's Illustrated Reviews Series)*. Hagerstwon, MD: Lippincott Williams & Wilkins. ISBN 0-7817-8215-5.

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