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# Novel food gelling agent as a topical probiotic delivery system

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A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

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by

Yamuna Gopan

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**Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Science in food innovation.**

Novel food gelling agent as a topical probiotic delivery system

by

Yamuna Gopan

Probiotics are certain living microorganisms that have to be  in the human body for health benefits. The administration of probiotics can treat different metabolic disorders and other health issues. Bacteria are sensitive to their environment. The viability of probiotics is affected by adverse conditions such as pH and temperature. Probiotic delivery can be in different modes. Nowadays, food products containing probiotic mixtures are of great interest by conumers. These products are for the administration of probiotics to improve the gut microflora. Oral administration of probiotics can improve not only the gut health, but it is a holistic approach of health improvement also. The immunomodulation ability of probiotics is beneficial for treatments of a number disorders and skin improvement. Objective of this research was to study the effects of a novel food gel(NG) on the viability of *Lactobacillus acidophilus* and antimicrobial activity against acne causing bacteria *Propionibacterium acnes*. An acne treatment ointment had taken as a control to compare the microbial analysis. The results showed, a negative effect of gel on the viability of *Lactobacillus acidophilus*, but a positive result was obtained for the antimicrobial activity of gel against *Propionibacterium acnes.* The flow characteristics were depended on the temperature and was suitable for a topical gel formulation. Further studies must be done for formulate new food gel as a topical acne treatment ointment.

**Keywords:** probiotics, probiotic delivery system, novel gel, cell viability, antimicrobial activity, *Lactobacillus acidophilus*, *Propionibacterium acnes*, flow characteristics.

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# Chapter1

# Introduction

Probiotics are of great interest nowadays because interaction between the microbiota and humans is studied and explained as beneficial to holistic health of human body. Probiotics maintains microbial gut balance and prevent and combat on pathogenic microbes and infections(Kobyliak et al., 2018). Administration of probiotics in a sufficient amount and as combinations of different bacteria are proved as efficient disease control strategies.

The stability and viability of probiotic bacteria are the important factor for their functionality in the animal system and it could be hindered by undesirable process conditions such as high temperature, oxygen, acidity, and humidity during the manufacture and storage. Moreover, probiotics are subjected to critical issues of low pH, bile salts, osmotic stresses, and oxidation while administered orally through the passage of gastrointestinal tract. These could affect the viability and thereby functionality of probiotics prior to reach the target site (D'Orazio et al., 2019).

To limit the loss of viability and to ensure stability and functionality, micro-encapsulation techniques are used for probiotic delivery. Nonetheless, the research on finding novel probiotic delivery systems is to be conducted which are biocompatible, more efficient and consumer friendly. Alginate based microcapsules are widely used as delivery agents, but these are sensitive to harsh acidic condition in gut region. The inability of these gelling polymers to withstand negative factors limit their use as encapsulating agent (D'Orazio et al., 2019). Not only the sensitivity of gel system, but also the antimicrobial activity of delivery agent is important to ensure safe and stable probiotic administration. Orally disintegrating films which act as bioactive compound carriers and mouth fresheners can also be suitable strategy for oral probiotic delivery. This can be a better alternative for capsules and tablets (Heinemann, Carvalho, & Favaro-Trindade, 2013). There are studies explaining the topical administration of probiotics for disease treatment. Topical probiotics application balances the skin microbiota and alleviate the symptoms and risks associated with the skin conditions due to immune responses like an anti-inflammatory drug or gel (Oelschlaeger, 2010).

Substances having viscoelastic nature especially polysaccharides or proteins used in foods for gelation are known as food gels or gelling agents. Due to the ability of forming three dimensional networks, these gels can be carriers of probiotic bacteria. This research investigates that, whether the novel gel prepared by combining citric acid (CA) and disodium 5-guanylate (DG) can be developed as a good probiotic delivery system or not.

## 1.2 Review of literature

### 1.2.1 Probiotics

Microorganisms that are essential for animal health and present in the gut region are commonly known as probiotics. These microorganisms are non-pathogenic but have protective function on the metabolism and physiology of the host. Administration of probiotic bacteria have been reported as effective treatments for different diseases. The colonisation of probiotic bacteria in animal gut can effectively prevent the colonisation of pathogenic microbes using the mechanism of competition as well as by producing bacteriocins against pathogens(Smith & Jones, 2012).

Modes of action of probiotics are mainly three include host immune modulation, direct action towards pathogens, and detoxification of host body (Oelschlaeger, 2010). Derivatives or cell components of probiotics can modulate the immune system of host by stimulating the cellular receptors in host gut epithelium.

Probiotics, which are formulated as food supplements contain yeast or beneficial gut bacteria. They are marketed as capsules or powders and are included in fermented food products mainly in dairy products such as yogurt and processed milk drinks. In probiotic products either a single probiotic bacteria or mixture of different species may present (Williams, 2010).

### *1.2.2 Lactobacillus acidophilus*

*L. acidophilus* is a probiotic bacterium that is extensively used in the production of dairy products and is widely applied in health sector, for improving immunity and boosting up the gut health. This bacterium is administered to humans and animals as a part of the diet in the form of dairy foods and dietary supplements. *L. acidophilus is* a potential vaccine-delivery agent to the gastrointestinal tract. The health improving effects of *L. acidophilus* include the treatment of diarrhoea, and other enteric diseases, diabetes treatment and balancing of gut microflora through the growth modulation mechanism in gastrointestinal tract. This bacterium was isolated from mouth as well as vaginal microflora also (Kim, Perl, Park, Tandianus, & Dunn, 2001).

The ability to adhere to the intestinal epithelium is a vital requirement for a probiotic bacterium to colonize and grow in the gastrointestinal tract which is important to prevent its peristaltic elimination and to adapt in the competitive environment. *L. acidophilus* is resistant to bile juice and resist digestion. *L. acidophilus* can aggregate themselves and have the ability of adhesiveness by the help of the proteinaceous cell surface component (Kos et al., 2003).

### 1.2.3 Probiotic delivery and matrices

probiotic delivery is important for the effective application of probiotic bacteria for alleviation of a disease condition. If probiotics are delivered efficiently to the target, only the administration will be successful. Generally, probiotic delivery systems are nutraceutical products which supplement the probiotic microorganisms to the intestinal environment. The delivery systems influence the effectiveness of treatment and health improvement of a patient. Therefore, selection of a probiotic delivery agent is important. There are different categories of probiotic delivery systems, which include conventional, pharmaceutical formulations, and non-conventional food-based products. The effectiveness of delivery system is based on the ability of delivering viable cells in large amount and the capacity to protect the cells from adverse components of gastric region(Govender et al., 2014).

Because of the extensive health benefits of probiotic bacteria, these are added to the food products especially the fermented milk products, ice creams, and dairy drinks. When the product is consumed, the bacteria should be viable and metabolically stable in the product, should pass through the digestive tract from the mouth, and should reach the target area, that is intestine. According to FAO/WHO, when food is manufactured as a probiotic delivery system it must contain at least $10^6$–$10^7$ cfu of viable probiotic bacteria per gram of food. However, the low viability of probiotics in dairy foods is common and is due to several factors. The fermentation requirements like titratable acidity, pH, dissolved oxygen, temperature, strains of microbes, and organic acids used for fermentation. Survival of the probiotic bacteria is the most essential thing for the provision of health benefits to human body(Anal & Singh, 2007). Food matrix with protective nature towards probiotic bacteria is reported as an alternative strategy for efficient delivery of bacteria(Possemiers, Marzorati, Verstraete, & Van de Wiele, 2010). A research study in 2007 reported that the lipid fraction of coca butter can be a protective delivery agent for bifidobacteria (Lahtinen, Ouwehand, Salminen, Forssell, & Myllärinen, 2007).

Encapsulation of probiotic bacteria is another mode of delivery which can prevent the exposure of bacteria to adverse process conditions and environments and can reduce the loss of microbial cells. Several researchers have reported their success in microencapsulation of bacteria with different matrices. For Lactic acid bacteria, alginate a non-toxic chemical is widely used to immobilize and deliver. Alginate is reported as a simple, cost effective, but effective encapsulating material(Sultana et al., 2000).

### 1.2.4 Topical administration of probiotics

Probiotics can be administered externally on skin also for the improvement of skin conditions. A study investigated the effects of ointment derived from *Lactobacillus reuteri* as a probiotic bacterial topical delivery system for the wound healing process. They used rat skin as a model and found that there is significant accelerating effect of probiotic derived ointment on wound healing (Khodaii, Afrasiabi, Hashemi, Ardeshirylajimi, & Natanzi, 2019).

Kefir cream which contains prebiotics and probiotics is reported as an effective topical ointment for the healing of dorsal lesions. A common inflammation of foreskin of dog's genital tract was also reported as treated when 70% kefir-lanette based ointment (Schneedorf, 2012).

Exogenous application of lactobacilli for the treatment of vaginal infection was studied as an alternative for the antimicrobial chemicals and drugs and it is reported that, this application can confer benefits to women for the vaginal infection(Reid, 2002).

### 1.2.5 Propionibacterium acnes and acne treatment

*Propionibacterium acnes* is a non-spore-forming, Gram-positive, pleomorphic bacteria which grow in anaerobic conditions. The end products of anaerobic fermentation of Propionibacterium include propionic acid. It is a common bacteria present in the normal microflora of oral cavity, large intestine, eyes, ear canal, and skin(Brook & Frazier, 1991). It has a major role in acne vulgaris, and associated with conditions sarcoidosis and synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO). *Propionibacterium acnes* can produce different virulence factors and leads to inflammation and allergic reactions(Perry & Lambert, 2006). Acne is a common skin problem which may worsen sometime. Acne treatment is widely conducted all over the world, but modes of treatments are different. Antibiotic therapy is the most common acne treatment which has specifically focused on *Propionibacterium acnes* elimination and has been active for more than 40 years. But the antibiotic resistance of *Propionibacterium acnes* against tetracyclines and erythromycin was reported from USA due to the widespread use. These findings enlightened the path of new strategies for the acne treatment (Eady, Gloor, & Leyden, 2003).

### 1.2.6 Food gels as probiotic delivery system

Polysaccharide hydrogels have extensive applications in various fields. polysaccharide-based hydrogels are common components of food products as thickeners and gelling agents. Due to the

sensitivity of probiotics to certain environments, encapsulating agents or coating materials are used as probiotic delivery systems. Polysaccharide hydrogels provide a physical barrier between probiotic bacterial cell and the adverse environment which enhance cell viability rate. Hydrogels can protect cells from heating process also. Alginate, κ-carrageenan, xanthan, pectin and chitosan are hydrogels used in food industry and can act as probiotic delivery agents(Kwiecień & Kwiecień, 2018).

## 1.3 Aims

This study aims mainly at the microbiological parameters of the novel gel, to find out whether the gel is suitable for the growth of *Lactobacillus acidophilus* and to investigate the antimicrobial activity of this new gel against *Propionibacterium acnes*. If the gel allows *Lactobacillus acidophilus* to grow, the gel can be developed into a probiotic delivery system. If there is any antimicrobial property against *Propionibacterium acnes* the gel can be efficiently applied in acne treatment.

## 1.4 Hypothesis

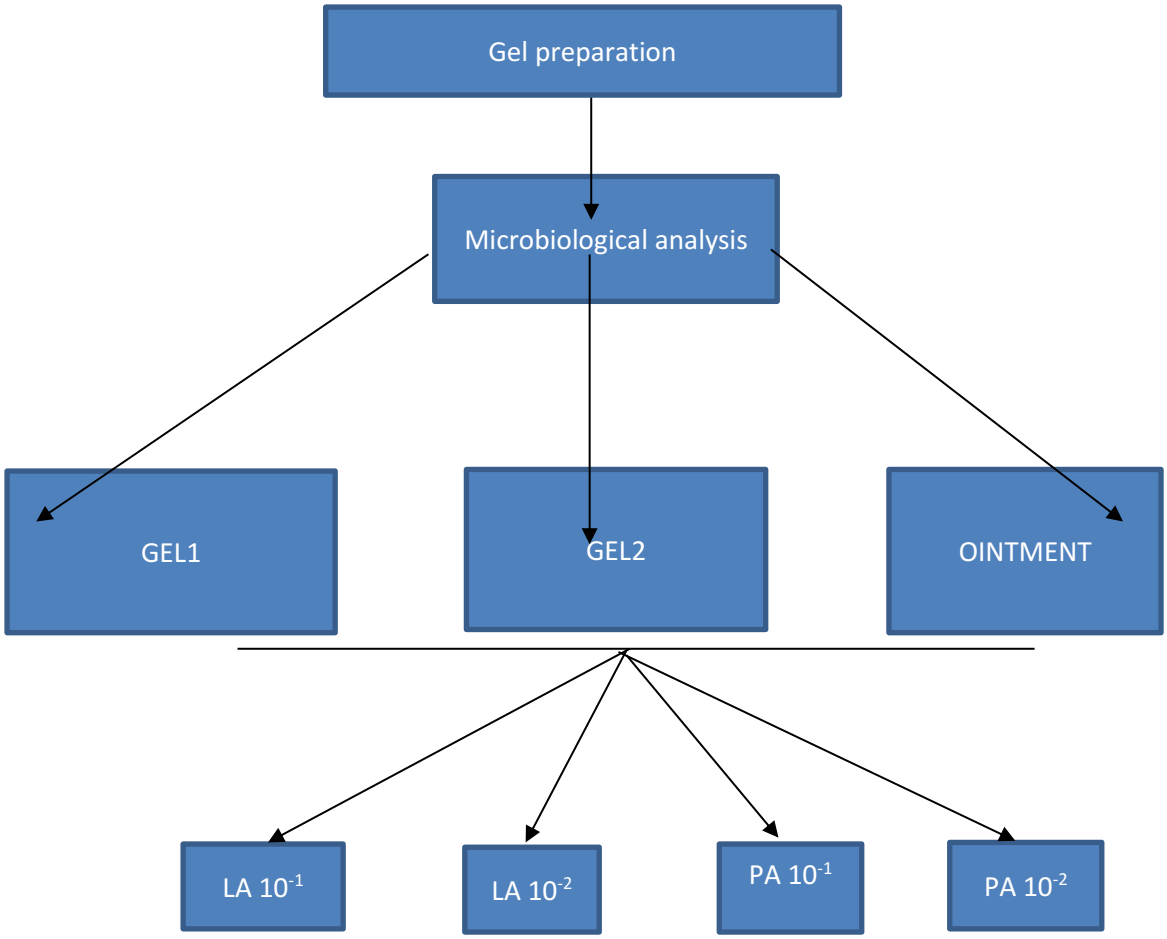
- Lactobacillus acidophilus will grow and will be viable in novel gel.
- There will be antimicrobial activity for the novel gel against *Propionibacterium acnes.*

# Chapter 2

# Materials and methods

## 2.1 Experimental design

### 2.1.1 Figure 2.1.1 experimental plan




*GEL1= 1M CA + 0.25M DG

*GEL2= 0.5M CA + 0.25M DG

*LA= *Lactobacillus acidophilus*

*PA= *Propionibacterium acnes*

## 2.2 Gel preparation

Two gels were prepared in different concentrations. Both gels contained same concentration of DG, only CA was differed. To prepare gels, DG and CA solutions were prepared in equal amount by dissolving in RO water separately. 0.25M concentration of DG, 1M, and 0.5 M citric acid solutions were prepared for two gels. The prepared DG was at 0.25 M concentration, CA at two concentrations such as 1M (gel1) and 0.5M (gel2). DG solution was mixed with two concentrations of CA to form two different gels. The mixture of CA and DG was mixed thoroughly using vortex for 15 seconds and kept in water bath for 10 minutes. These two gels were used for the microbiological experiments, and the gel 2 was used for flow characteristics analysis, as it appeared to be thin and good consistency.

Benzac AC from Galderma Australia private Ltd 60g(2.5%) ointment was purchased from Wecare pharmacy, Lincoln and used a control for the microbial analysis. It is an aid in the acne treatment.

**2.2.1** Table 2.2.1 showing the composition of samples and controls.

| **SAMPLES** | **COMPOSITION** |
|---|---|
| T1 | GEL1+ ($10^{-1}$) LA / PA |
| T2 | GEL1 + ($10^{-2}$) LA / PA |
| T3 | GEL2 + ($10^{-1}$) LA / PA |
| T4 | GEL2 + ($10^{-2}$) LA / PA |
| C1 | OINTMENT + ($10^{-1}$) LA / PA |
| C2 | OINTMENT + ($10^{-2}$) LA / PA |
| C3 | MRS/BLOOD AGAR WITHOUT CULTURE |

## 2.3 Experimental set up of microbiological analysis

### 2.3.1 Viability of *Lactobacillus acidophilus* in gel

Gels in room temperature were heated up to 37°C using water bath and 950µL of gel was taken in 1ml Eppendorf tube. Eppendorf tubes with 950 µL gel were prepared for all samples for analysis. Two dilutions of *Lactobacillus acidophilus* culture were prepared ($10^{-1}$,$10^{-2}$) using 9ml sterile 0.1% (w/v) peptone water. 50 µL bacterial dilutions was added to the respective tubes with gel and tubes were vortexed until the bacterial culture and gel were mixed and kept for incubation at 37°C for 1 hour. De Man, Rogosa, and Sharpe (MRS) agar plates received from Fort Richards Laboratories limited, Auckland, New Zealand were used for doing spread plate method for bacterial colony formation. 100 µL gel-bacteria mixture were poured and spread on MRS agar plates using sterile disposable L-rods. Spread plates were incubated at $37^0$C for 48 hours under anaerobic condition (Dave & Shah, 1996). After 48 hours, colonies were counted, and CFU/ml was calculated.

### *2.3.2* Antimicrobial activity of novel gel against *Propionibacterium acnes*

950 µL gel was taken in 1 ml Eppendorf tube. Two dilutions of *Propionibacterium acnes* were prepared ($10^{-1}$,$10^{-2}$) using 9ml sterile 0.1% (w/v) peptone water. The initial culture of *Propionibacterium acnes* was obtained from ESR was used for the experiment. 50 µL bacterial dilutions was added to the properly labelled tubes with gel and tubes were vortexed until the bacterial culture and gel were mixed. Blood agar for the analysis was purchased from Fort Richards Laboratories ltd, Auckland, New Zealand. 100 µL gel-bacteria mixture were poured and spread on blood agar plates using sterile disposable L-rods and the plates were incubated at $37^0$C for 48 hours under anaerobic condition. Colonies were counted after 48 hours and CFU/ml was calculated.

## 2.4 Flow characteristics

The rheological properties of gel2 (0.25DG+0.5 CA) in 22°C and 4°C were measured to characterize flow properties of gel. For the rheology analysis a concentric cylinder viscometer was used which was made of an inner co-axial cylinder which was rotating within a stationary cylinder. Viscosity measurements were taken at shear rates from 19.3 to 161$s^{-1}$ for 60 seconds. Gel 2 kept in 4°C and kept in water bath at 22°C for 10 minutes were used for the analysis.

## 2.5 Statistical analysis

Mean and standard deviation of CFU/ml were calculated using Microsoft Excel and graphs also plotted using it. Statistical analysis, ANOVA was performed using Minitab 2019 version. Tukey comparison was done to evaluate significant differences between the samples.

# Chapter 3

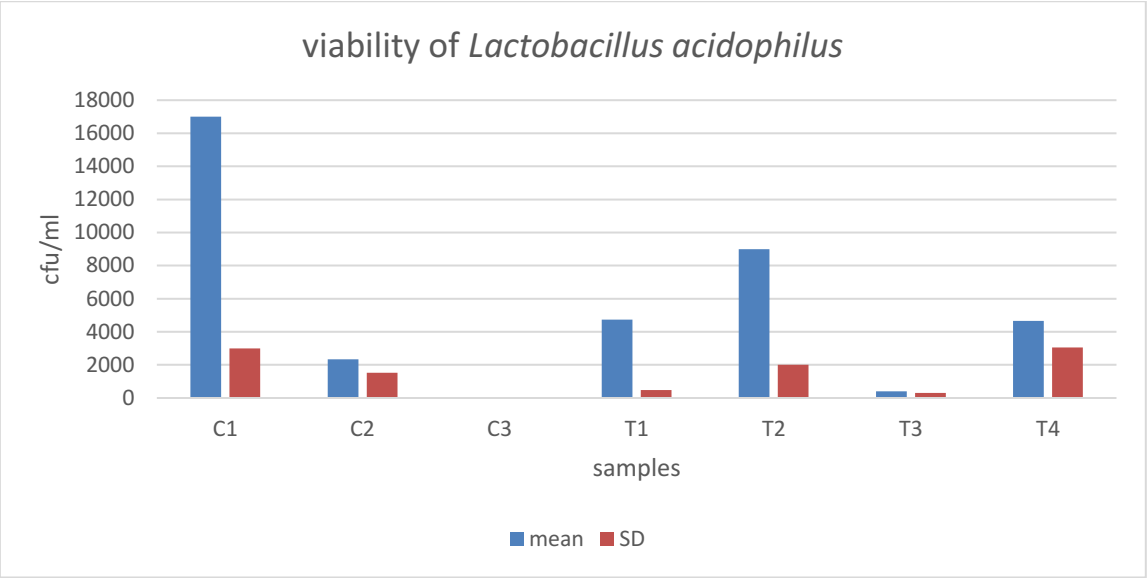
# Results and discussion

## 3.1 Viability of *Lactobacillus acidophilus*

Enumeration of *Lactobacillus acidophilus* has done to find out the effect of novel food gel on the viability of the probiotics. The counts of colonies in MRS agar were converted to CFU/ mL and a graph was plotted to show the difference in different samples. All the samples were done in triplicates.

### 3.1.1 Table3.1.1

| sample | mean | SD |
|---|---|---|
| **C1** | 17000 | 3000 |
| **C2** | 2333 | 1528 |
| **C3** | 0 | 0 |
| **T1** | 4733 | 473 |
| **T2** | 9000 | 2000 |
| **T3** | 400 | 300 |
| **T4** | 4667 | 3055 |



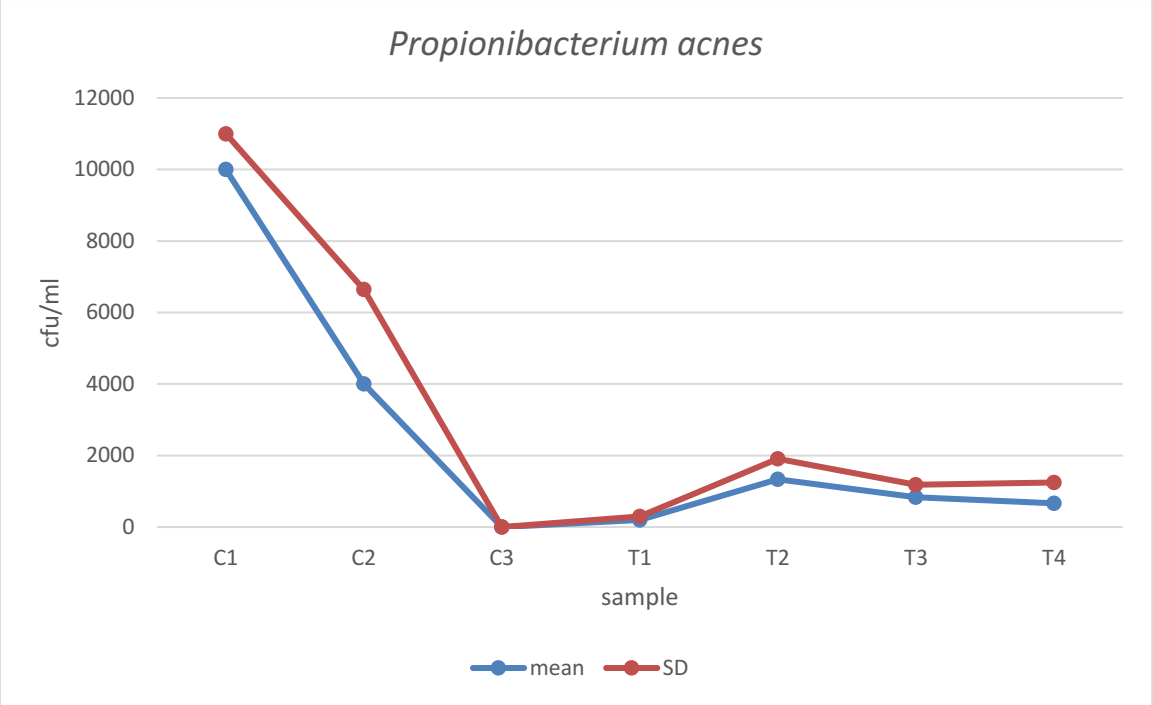

### *3.1.2* Figure3.1.1 graph showing the mean and standard deviation cfu of *Lactobacillus acidophilus.*

C1 is the control ointment with $10^{-1}$ *L. acidophilus* dilution and CFU/ml was highest in C1 control. T2 was the sample with Gel1 and $10^{-2}$ dilution which showed the second higher CFU/ml value. T1 and T4 showed almost same CFU/ml value in which the former was gel1with $10^{-1}$ and the latter was gel2 with $10^{-2}$ dilution. In sample T3 with gel2 and $10^{-1}$ dilution the CFU/ml value was the lowest.

From the statistical analysis, it could be clearly observed that, the CFU/ml were significantly different among the 4 samples and 3 controls as the $p<0.05$. From these results, the gel samples showed relatively low CFU values compared to the C1 ointment control. This indicated any gel component can be the reason behind the low viability of cells in gel. A previous study on a DNA-based complex gel used for microencapsulation reported that, the starch and protein fraction of the gel composition might be provided a favourable environment for bacterial growth and even in adverse condition made up by neutral simulated intestinal juice. Therefore, the DNA-based complex gel can be a protective covering to the bacteria(Jonganurakkun, Liu, Nodasaka, Nomizu, & Nishi, 2003), but in this study an opposite effect has occurred. Another study of microencapsulation using hydrogel beads, also reported the survival of the encapsuled lactobacillus plantarum cells at 72°C for 9seconds during juice processing, the hydrogel was a hybrid gel of alginate and soy protein isolate(Praepanitchai, Noomhorm, & Anal, 2019), this study also reported that, unencapsulated cells died at 50°C. This indicated that reason behind the low viability of cells in gel can be either the cells did not entrap in the gel networks or effect of any of the components of the novel gel. When talking about Citric acid in the gel, citric acid has antimicrobial effect on few bacteria like *E.coli(In*, Kim, Kim, & Oh, 2013), but citrate is required for the metabolism of lactic acid bacteria(Drinan, Robin, & Cogan, 1976) and *L. acidophilus* is capable of surviving in acidic conditions(Sinn et al., 2008).

### 3.2 Antimicrobial effect of gel on *Propionibacterium acnes*

The enumeration of propionibacterial colony on blood agar was conducted for investigating the antimicrobial effect of novel gel on *Propionibacterium acnes*.




### 3.1.3 Figure 3.2.1 graph showing the mean and standard deviation of CFU/ml in different samples.

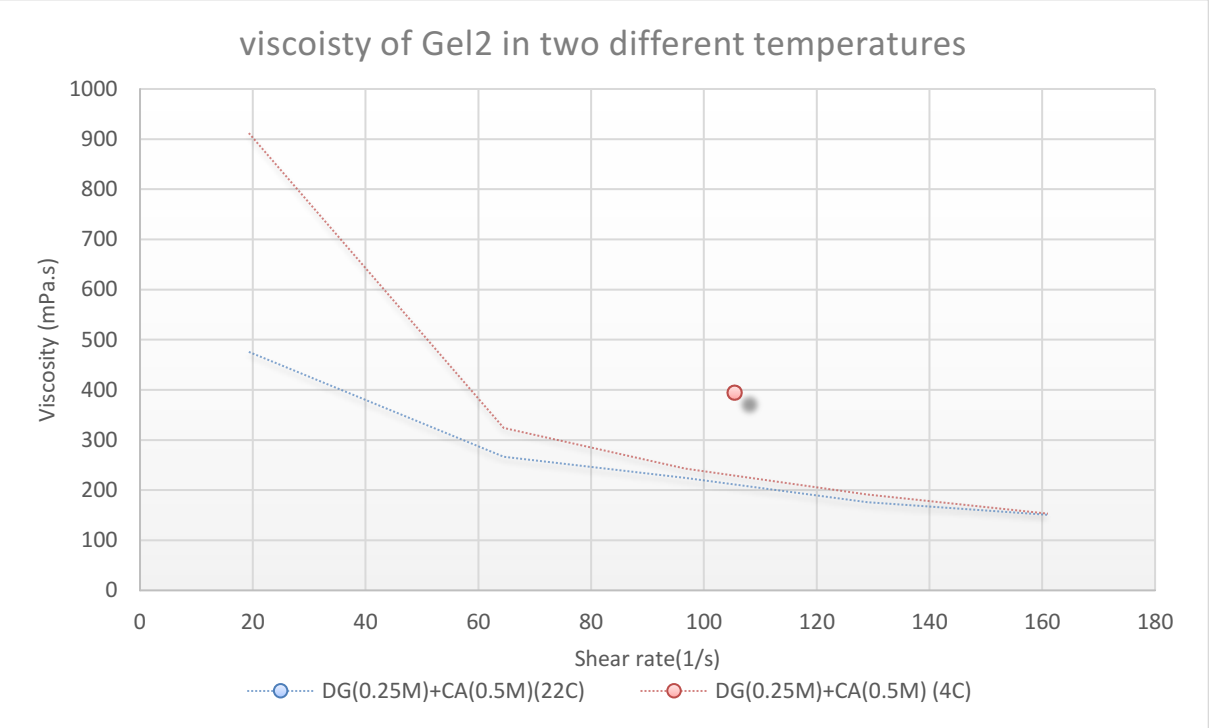
The graph shows the highest CFU value for the control C1 with ointment and $10^{-1}$ *P.acnes* dilution. The control C2 with ointment with $10^{-2}$ dilution shows second higher CFU value. T1 which is the Gel1 with $10^{-1}$ dilution showed the least CFU value, T3 and T4 showed almost same CFU values. T2 sample showed higher CFU than T3 and T4.

The statistics depicts that, there is a significant difference in CFU values among the samples and controls ($p<0.05$).

The highest CFU value of *P.acnes* in the controls of ointments showed the ineffectiveness of the ointment against *P.acnes.* All gel samples showed relatively very less CFU of *P.acnes* in the experiment. This is a positive result for the novel gel as it has an antimicrobial effect against pathogenic bacteria. This result is comparable with a study done on Indian herbal plants with

antimicrobial effect against acne inducing bacteria, because the plant origin of DG can be a reason for the antimicrobial effect (Kumar et al., 2007)as well as the antimicrobial effect of citric acid against *P.acnes* (Bae & Park, 2016). A study of antimicrobial effect of marine microalgae fractions, it is reported that, water soluble fractions and fat-soluble fractions showed above 90% antimicrobial activity against pathogenic bacteria(S.-K. Kim et al., 2001). This supports the antimicrobial activity of DG in gel against pathogens, as it is a water-soluble fraction of marine microalgae. A previous study on the novel gel for shelf-life study of meat products reported, the novel gel confer antimicrobial activity against certain bacteria and extended shelf life(Chelikani et al., 2021).

## 3.3 Flow characteristics




### 3.3.1 Figure 3.3.1 graph showing the flow characteristics of gel2 at 22°c and 4°c.

the effect of temperature influences the flow characteristics of the gel. Gel2 (DG-0.25M + CA 0.5M) was prepared and kept under 4° C and compared with gel at 22° C. the results showed significant difference in flow characteristics between the gels at two temperatures. Gel at 4° C showed higher viscosity at low shear rate. Gel at 22°C also showed the same trend. Both gels became less viscous when the shear rate increased. In high temperature, viscosity of gel is less. A relatable results can be seen in a research paper, shows that cooking temperature reduced the viscosity of carrageenan (Bono, Anisuzzaman, & Ding, 2014).

# Chapter 4

# Conclusion

Probiotic delivery agent has significant role in the effective administration and efficiency of probiotic to confer health benefits to human body. Studies reported that skin ailments can be treated by administrating single or mixture of probiotics orally or exogenously. Lot of acne treatment ointments and therapeutics are available in the market, but most of them are antibiotic based or antimicrobial chemical-based ointments. A better alternative for the antibiotic therapy is application of probiotics. This study aimed to prove that; the novel gel can be formulated as a topical probiotic delivery agent.

In this present study, the viability of *Lactobacillus acidophilus* in the novel gel prepared by Disodium 5- guanylate and Citric acid, the antimicrobial activity of the gel against *Propionibacterium acnes*, and the flow characteristics of the gel were analysed. The result showed, a negative effect of gel on the viability of *Lactobacillus acidophilus*, but a positive result was obtained for the antimicrobial activity of gel against *Propionibacterium acnes.* The flow characteristics were depended on the temperature and was suitable for a topical gel.

This study will give an insight for further research on the application of novel gel for the treatment of broad spectrum of bacterial infections. A major drawback in this study that, we did not try to analyse the effect of gel on microorganisms after a long-term storage.

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# Appendix A

# Primary data

## A.1 Composition of samples for microbiological analysis

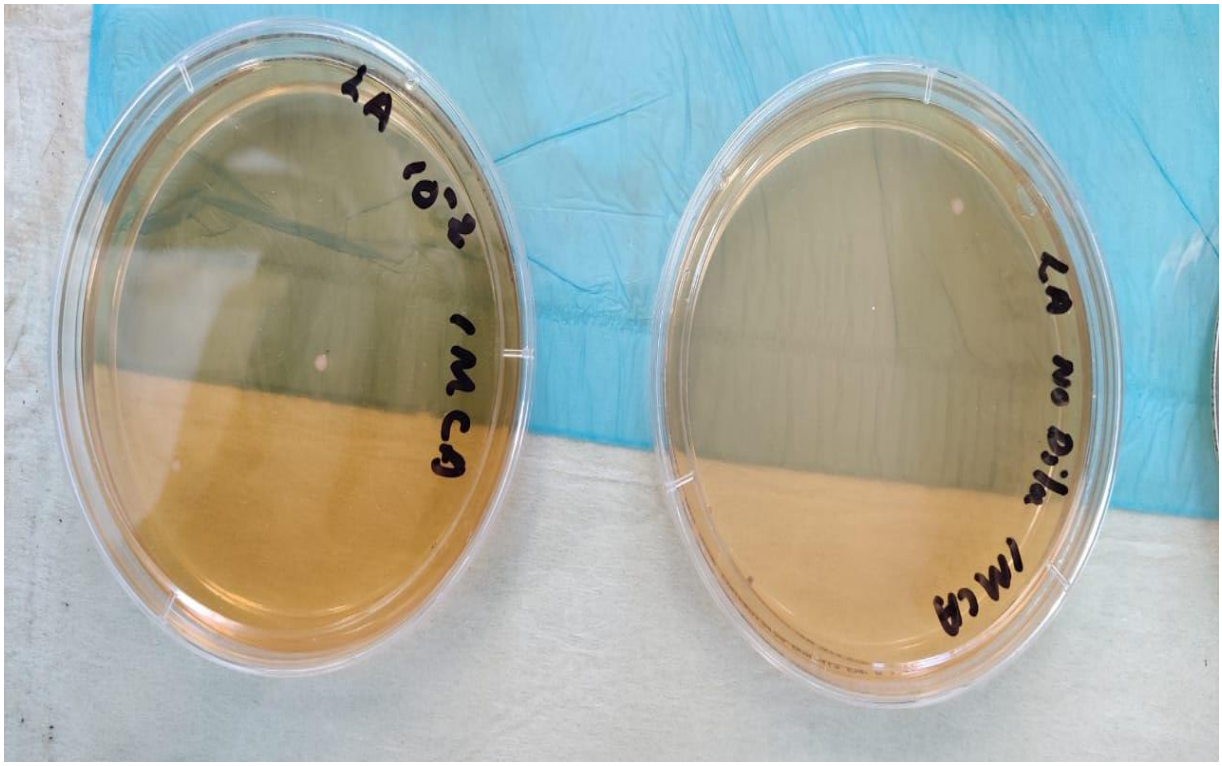
| SAMPLES | COMPOSITION |
|---|---|
| T1 | GEL1+ ($10^{-1}$) LA / PA |
| T2 | GEL1 + ($10^{-2}$) LA / PA |
| T3 | GEL2 + ($10^{-1}$) LA / PA |
| T4 | GEL2 + ($10^{-2}$) LA / PA |
| C1 | OINTMENT + ($10^{-1}$) LA / PA |
| C2 | OINTMENT + ($10^{-2}$) LA / PA |
| C3 | MRS/BLOOD AGAR WITHOUT CULTURE |

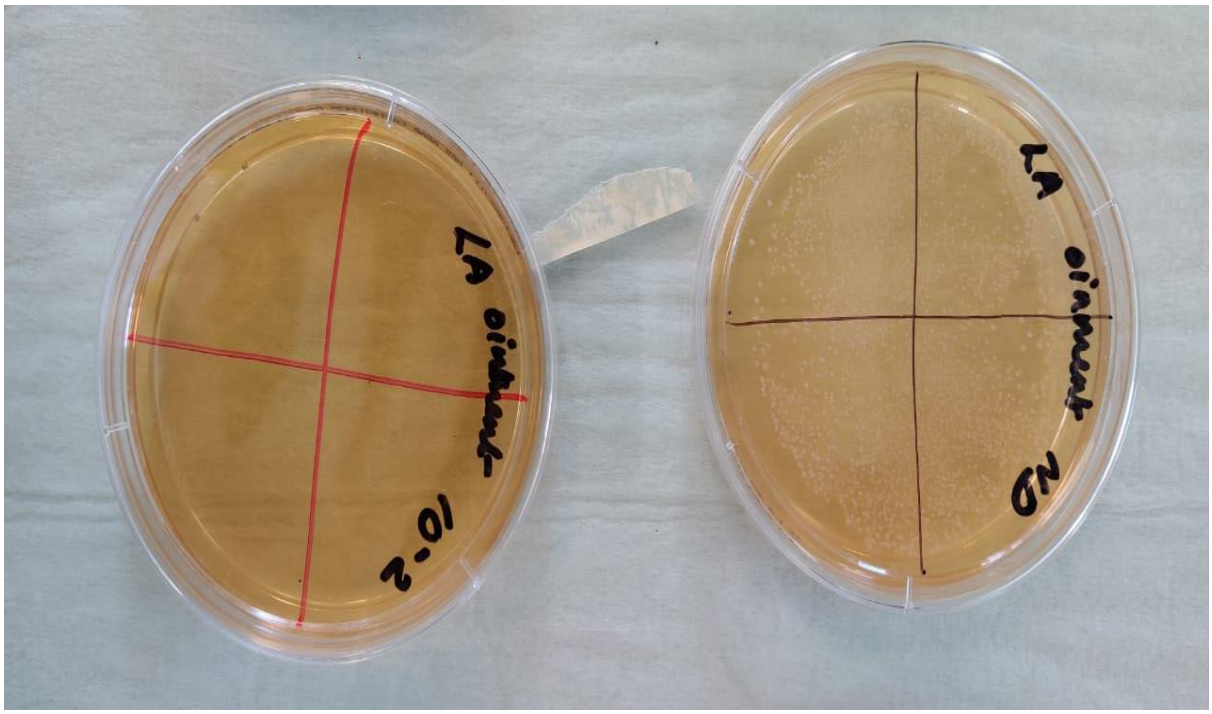
## A.2 Viability of *Lactobacillus acidophilus*

### CFU/ml in MRS agar plate

| SAMPLE | CFU |
|---|---|
| **T1** | 4200 |
| **T1** | 5100 |
| **T1** | 4900 |
| **T2** | 7000 |
| **T2** | 9000 |
| **T2** | 11000 |
| **T3** | 100 |
| **T3** | 400 |
| **T3** | 700 |
| **T4** | 2000 |
| **T4** | 8000 |
| **T4** | 4000 |
| **C1** | 17000 |
| **C1** | 20000 |
| **C1** | 14000 |
| **C2** | 4000 |
| **C2** | 1000 |
| **C2** | 2000 |
| **C3** | 0 |
| **C3** | 0 |
| **C3** | 0 |

**A 2.1 *Lactobacillus acidophilus* colony visualization on MRS agar**



**Descriptive Statistics: CFU**

## Statistics

| Variable | SAMPLE | N | N* | Mean | SE Mean | StDev | Minimum | Q1 | Median | Q3 |
|---|---|---|---|---|---|---|---|---|---|---|
| CFU | C1 | 3 | 0 | 17000 | 1732 | 3000 | 14000 | 14000 | 17000 | 20000 |
| | C2 | 3 | 0 | 2333 | 882 | 1528 | 1000 | 1000 | 2000 | 4000 |
| | C3 | 3 | 0 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| | T1 | 3 | 0 | 4733 | 273 | 473 | 4200 | 4200 | 4900 | 5100 |
| | T2 | 3 | 0 | 9000 | 1155 | 2000 | 7000 | 7000 | 9000 | 11000 |
| | T3 | 3 | 0 | 400 | 173 | 300 | 100 | 100 | 400 | 700 |
| | T4 | 3 | 0 | 4667 | 1764 | 3055 | 2000 | 2000 | 4000 | 8000 |

| Variable | SAMPLE | Maximum |
|---|---|---|
| CFU | C1 | 20000 |
| | C2 | 4000 |
| | C3 | 0.000000 |
| | T1 | 5100 |
| | T2 | 11000 |
| | T3 | 700 |
| | T4 | 8000 |

**General Linear Model: CFU versus SAMPLE**

## Method

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

## Factor Information

| Factor | Type | Levels | Values |
|---|---|---|---|
| SAMPLE | Fixed | 7 | C1, C2, C3, T1, T2, T3, T4 |

## Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| SAMPLE | 6 | 636152381 | 106025397 | 29.71 | 0.000 |
| Error | 14 | 49960000 | 3568571 | | |
| Total | 20 | 686112381 | | | |

## Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 1889.07 | 92.72% | 89.60% | 83.62% |

## Coefficients

| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 5448 | 412 | 13.22 | 0.000 | |
| SAMPLE | | | | | |
| C1 | 11552 | 1010 | 11.44 | 0.000 | 1.71 |
| C2 | -3114 | 1010 | -3.08 | 0.008 | 1.71 |
| C3 | -5448 | 1010 | -5.40 | 0.000 | 1.71 |
| T1 | -714 | 1010 | -0.71 | 0.491 | 1.71 |
| T2 | 3552 | 1010 | 3.52 | 0.003 | 1.71 |
| T3 | -5048 | 1010 | -5.00 | 0.000 | 1.71 |

## Regression Equation

CFU = 5448 + 11552 SAMPLE_C1 - 3114 SAMPLE_C2
- 5448 SAMPLE_C3 - 714 SAMPLE_T1
+ 3552 SAMPLE_T2 - 5048 SAMPLE_T3
- 781 SAMPLE_T4

## Fits and Diagnostics for Unusual Observations

| Obs | CFU | Fit | Resid | Std Resid | |
|---|---|---|---|---|---|
| 11 | 8000 | 4667 | 3333 | 2.16 | R |

*R Large residual*

**Comparisons for CFU**

## Tukey Pairwise Comparisons: SAMPLE

## Grouping Information Using the Tukey Method and 95% Confidence

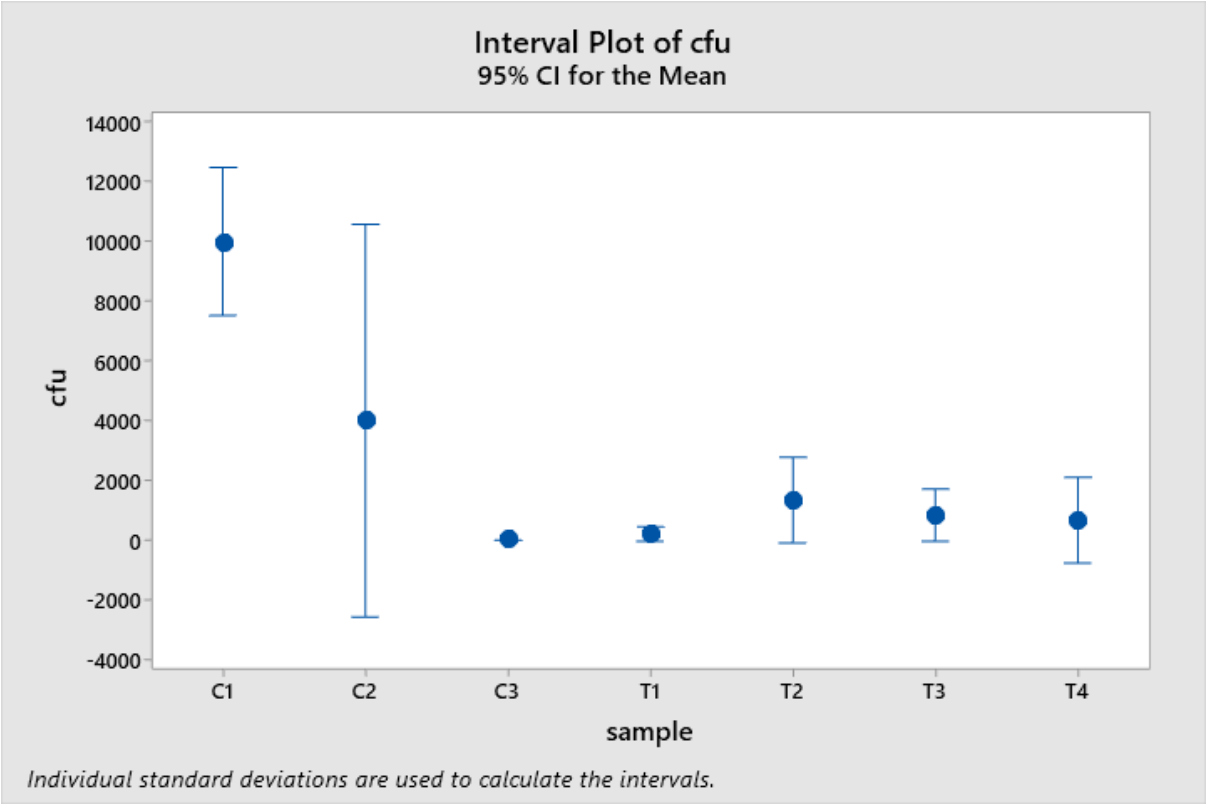
| SAMPLE | N | Mean | Grouping | | |
|---|---|---|---|---|---|
| C1 | 3 | 17000.0 | A | | |
| T2 | 3 | 9000.0 | | B | |
| T1 | 3 | 4733.3 | | B | C |
| T4 | 3 | 4666.7 | | B | C |
| C2 | 3 | 2333.3 | | | C |
| T3 | 3 | 400.0 | | | C |
| C3 | 3 | 0.0 | | | C |

*Means that do not share a letter are significantly different.*

## A3 Antimicrobial activity against *Propionibacterium acnes*

### A3.1 CFU in blood agar

| Sample | cfu |
|---|---|
| T1 | *200* |
| T1 | *100* |
| *T1* | *300* |
| *T2* | *2000* |
| *T2* | *1000* |
| *T2* | *1000* |
| *T3* | *500* |
| *T3* | *800* |
| *T3* | *1200* |
| *T4* | *1000* |
| *T4* | *1000* |
| *T4* | *0* |
| *C1* | *10000* |
| *C1* | *11000* |
| *C1* | *9000* |
| *C2* | *5000* |
| *C2* | *1000* |
| *C2* | *6000* |
| *C3* | *0* |
| *C3* | *0* |
| *C3* | *0* |




**Descriptive Statistics: cfu**

## Statistics

| Variable | sample | N | N* | Mean | SE Mean | StDev | Minimum | Q1 | Median | Q3 |
|---|---|---|---|---|---|---|---|---|---|---|
| cfu | C1 | 3 | 0 | 10000 | 577 | 1000 | 9000 | 9000 | 10000 | 11000 |
| | C2 | 3 | 0 | 4000 | 1528 | 2646 | 1000 | 1000 | 5000 | 6000 |
| | C3 | 3 | 0 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| | T1 | 3 | 0 | 200.0 | 57.7 | 100.0 | 100.0 | 100.0 | 200.0 | 300.0 |
| | T2 | 3 | 0 | 1333 | 333 | 577 | 1000 | 1000 | 1000 | 2000 |
| | T3 | 3 | 0 | 833 | 203 | 351 | 500 | 500 | 800 | 1200 |
| | T4 | 3 | 0 | 667 | 333 | 577 | 0 | 0 | 1000 | 1000 |

| Variable | sample | Maximum |
|---|---|---|
| cfu | C1 | 11000 |
| | C2 | 6000 |
| | C3 | 0.000000 |
| | T1 | 300.0 |
| | T2 | 2000 |
| | T3 | 1200 |
| | T4 | 1000 |

WORKSHEET 2

**General Linear Model: cfu versus sample**

## Method

| | |
|---|---|
| Factor coding | (-1, 0, +1) |

## Factor Information

| Factor | Type | Levels | Values |
|---|---|---|---|
| sample | Fixed | 7 | C1, C2, C3, T1, T2, T3, T4 |

## Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| sample | 6 | 232526667 | 38754444 | 30.83 | 0.000 |
| Error | 14 | 17600000 | 1257143 | | |
| Total | 20 | 250126667 | | | |

## Model Summary

| S | R-sq | R-sq(adj) | R-sq(pred) |
|---|---|---|---|
| 1121.22 | 92.96% | 89.95% | 84.17% |

## Coefficients

| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 2433 | 245 | 9.95 | 0.000 | |
| sample | | | | | |
| C1 | 7567 | 599 | 12.63 | 0.000 | 1.71 |
| C2 | 1567 | 599 | 2.61 | 0.020 | 1.71 |
| C3 | -2433 | 599 | -4.06 | 0.001 | 1.71 |
| T1 | -2233 | 599 | -3.73 | 0.002 | 1.71 |
| T2 | -1100 | 599 | -1.84 | 0.088 | 1.71 |
| T3 | -1600 | 599 | -2.67 | 0.018 | 1.71 |

## Regression Equation

cfu = 2433 + 7567 sample_C1 + 1567 sample_C2
- 2433 sample_C3 - 2233 sample_T1
- 1100 sample_T2 - 1600 sample_T3
- 1767 sample_T4

## Fits and Diagnostics for Unusual Observations

| Obs | cfu | Fit | Resid | Std Resid | |
|---|---|---|---|---|---|
| 17 | 1000 | 4000 | -3000 | -3.28 | R |
| 18 | 6000 | 4000 | 2000 | 2.18 | R |

*R  Large residual*

**Comparisons for cfu**

**Tukey Pairwise Comparisons: sample**

**Grouping Information Using the Tukey Method and 95% Confidence**

| sample | N | Mean | Grouping | | |
|---|---|---|---|---|---|
| C1 | 3 | 10000.0 | A | | |
| C2 | 3 | 4000.0 | | B | |
| T2 | 3 | 1333.3 | | B | C |
| T3 | 3 | 833.3 | | | C |
| T4 | 3 | 666.7 | | | C |
| T1 | 3 | 200.0 | | | C |
| C3 | 3 | 0.0 | | | C |

*Means that do not share a letter are significantly different.*

**A3.2 *Propionibacterium acnes* colony visualization on blood agar**