

Good Bacteria Gone Rogue

Investigating the Antimicrobial Effects of *Lactobacillus Acidophilus* metabolites against

Staphylococcus epidermidis

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Abstract

Could the key to healthier skin lie in probiotics instead of traditional medicine? Acne affects millions of people worldwide, with *Staphylococcus epidermidis* playing a major role in breakouts, even though it is found on healthy, clear skin. As antibiotic resistance rises, new approaches are needed to control harmful bacteria. This experiment explored whether metabolites from *Lactobacillus acidophilus*, a “good bacteria” found in the human gut and on the surface of the skin, could inhibit the growth of *S. epidermidis*. Bacterial cultures were grown in control and test environments, with the test environments containing *L. acidophilus* metabolites. The growth was quantified through OD600 and CFU/mL. Differences in the bacterial growth showed that probiotic metabolites could reduce harmful bacteria activity. These results suggest that natural, probiotic treatments may offer promising alternatives for managing acne.

Purpose

The purpose of this experiment is to discover whether metabolites in *Lactobacillus acidophilus* can inhibit the growth of *Staphylococcus epidermidis*, an acne forming bacteria. Acne is a common skin condition that can cause inflammation and irritation, and finding natural or probiotic based methods to decrease the amount of acne-causing bacteria may provide more safer treatments.

Hypothesis

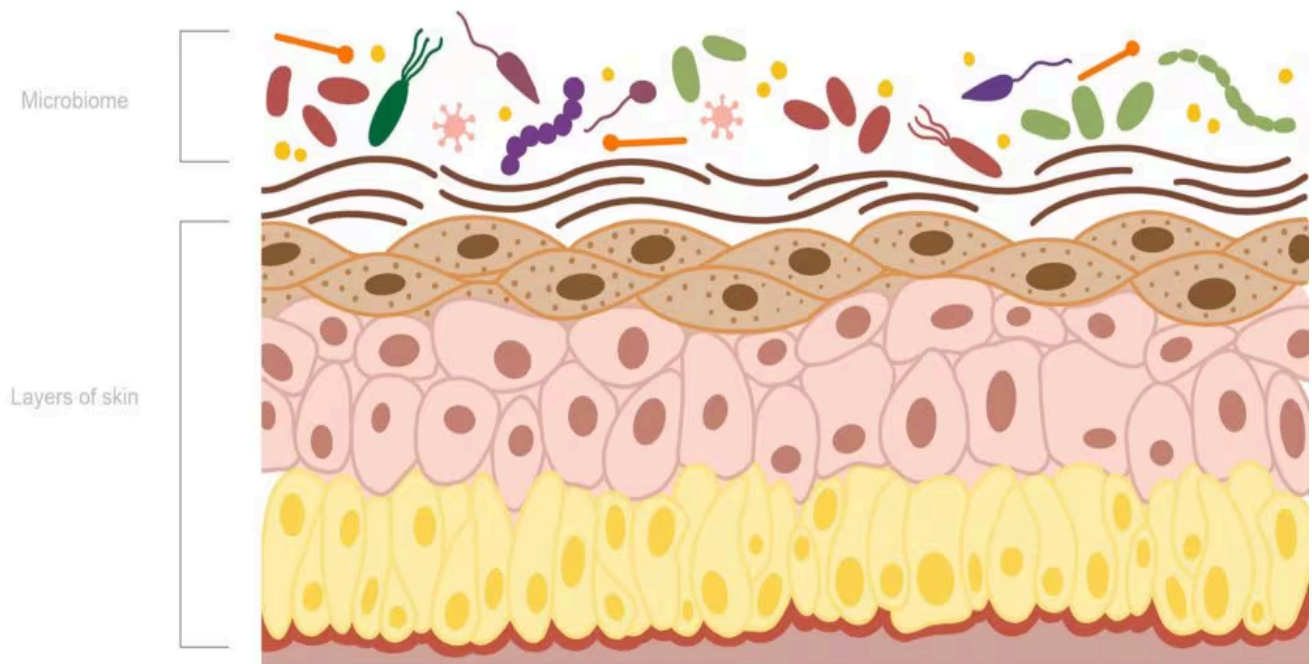
If *S. epidermidis* is cultured with *Lactobacillus acidophilus* metabolite solution in LB broth and another solution with only LB broth, then the bacteria exposed to *Lactobacillus*

acidophilus will have less growth because the lactic acid in the metabolites will deter the the growth of *S. epidermidis*.

Background Research

Skin Microbiome

The skin microbiome is the community of trillions of microorganisms, such as mites, fungi, bacteria, and viruses, that naturally live on the human skin. The skin is the human body's largest organ and is home to a diverse range of microbes (Wiginton, 2025). These microbes grow, reproduce, and adapt to specific skin conditions, whether it be oily, dry or moist (Grice & Serge, 2013). The microbiome fights off infections, where certain bacteria behave like antibiotics and decrease the pH of the skin, helping the immune system by warning the brain of any viruses present, and helps maintain inflammation and heal injuries (Wiginton, 2025).



The skin microbiome is made of bacteria, fungi and viruses that cohabitate on the top layer of skin.

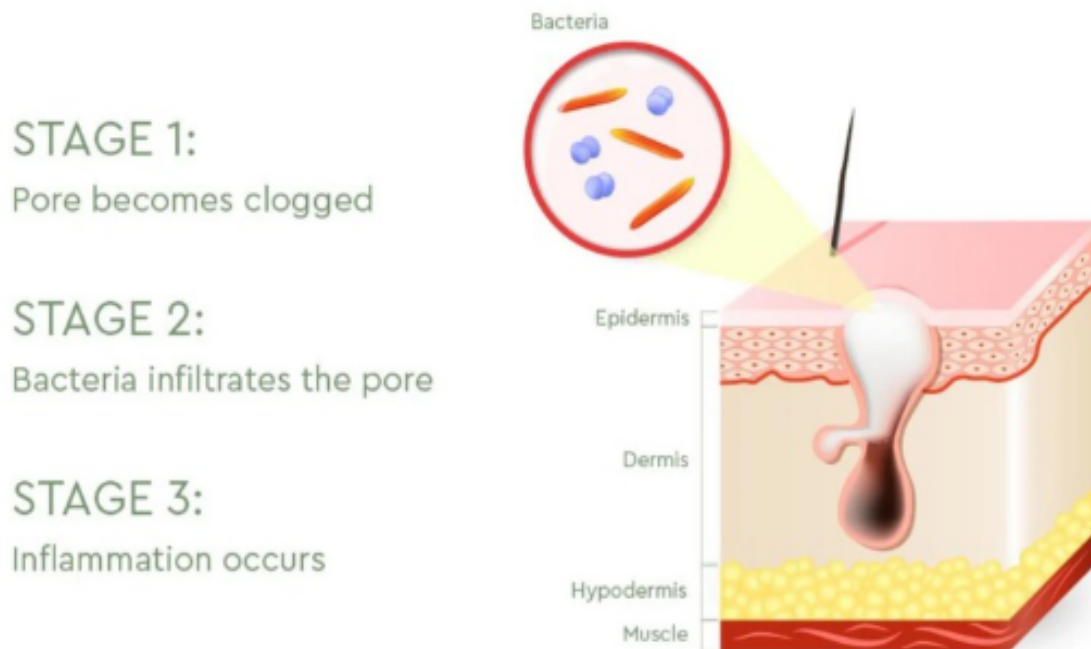
Most of the microorganisms present on the skin surface are beneficial or harmless to an individual. However, if the balance between good and bad bacteria is disturbed, known as dysbiosis, the human host may be more susceptible to skin diseases such as eczema, acne, or rosacea (Grice & Serge, 2013). An example of a bacteria that may do this is *S. epidermidis*. *S. epidermidis* is harmless when balanced on the skin. However, in large amounts, it can aid the growth of acne and trigger inflammation (Lee & Anjum, 2023). This example shows that skin health depends on maintaining a balanced microbial community rather than eliminating bacteria entirely. This is also why sterilization is avoided. This process may reduce the amount of harmful bacteria, but also decreases the population of the good bacteria (Grice & Serge, 2013). Conversely, holistic approaches to acne management include supporting the skin barrier by moisturizing regularly, and assisting the skin-gut-microbiome connection by balancing the diet and exercising consistently (Wiginton, 2025). However, holistic approaches are not sufficient to manage the condition in all individuals. As a result, treatments that maintain the natural balance of the skin microbiome are in high demand (Wiginton, 2025).

Acne

Acne is a skin condition in which dead skin cells and skin oil block hair follicles. In fact, 83% of girls and 95% of boys at the ages of 15-16 experience it, and many adults also struggle with acne (Chilicka et al., 2022). It can appear in many areas of the skin, including on one's face, shoulders, and chest. There are 4 main factors that contribute to acne growth (*Acne: Risk Factors*, n.d.):

1. Follicular hyperkeratinization: Hair follicles blocked by dead skin cells and sebum. This plug creates a breeding ground for bacteria to thrive in.

2. Increase in sebum production: Sebaceous glands expand in size and produce more sebum, especially during puberty. This sebum increases the chances of blockages if not properly cleared.
3. Growth of *C. acnes*: *Cutibacterium acnes*, or *C. acnes*, is a bacteria on healthy human skin. The sebum is the source of its nutrients. An increase of *C. acnes* and *S. epidermidis* can favour an increase of inflammation on the skin. Since there is more growth of *C. acnes*, bacteria fight for nutrients and to survive, leading to a further disbalance on the skin microbiome.
4. Inflammation: May cause follicles to rupture, creating red pimples. This red pimple is also known as a “tender”.



Antimicrobial Resistance

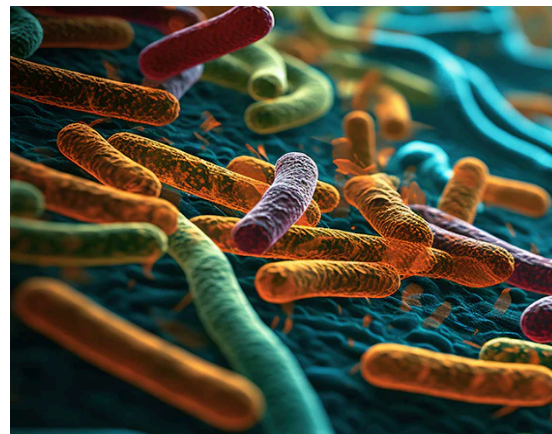
Antibiotic resistance is a growing health concern worldwide (Dessinioti & Katsambas, 2022). This occurs when a bacteria or disease adapts and becomes immune to the medication,

making it harder to treat, an issue that has also arisen in acne medications (Dessinioti & Katsambas, 2022), where skin bacteria adapt and find other ways to thrive in harsh conditions.

C. acnes is a type of bacteria found on healthy human skin, but may contribute to acne when present in large amounts. Antibiotics have been used to treat it, however, overuse of medication has led to the adaptation of certain bacteria, including *C. acnes* (Dessinioti & Katsambas, 2022). Additionally, antibiotics such as erythromycin and clindamycin disturb the natural balance of the skin microbiome. A balanced amount of bacteria can keep viruses or harmful bacteria from overgrowing (Dessinioti & Katsambas, 2022). Antibiotics affect that and put the skin at more risk (Dessinioti & Katsambas, 2022).

Lactobacillus Acidophilus

Lactobacillus acidophilus is a rod-shaped, gram-positive bacterium found in the human digestive system and is commonly used as a probiotic to promote healthy gut bacteria, supporting both immune and digestive functions (Definition of Lactobacillus - NCI Dictionary of Cancer Terms - NCI, n.d.). It produces lactic acid,



which helps maintain an acidic environment that inhibits harmful bacteria in the gut (Batt, 2014) and, when applied to the skin, dissolves dead skin cells, hydrates the skin barrier, and limits cell buildup (Batt, 2014). Additionally, *L. acidophilus* produces hydrogen peroxide, which further deters the growth of certain bacteria (Batt, 2014). When lactic acid is applied or produced on the

skin, it lowers the skin's pH and removes dead skin cells, which hydrates the skin, as shown in the study *Epidermal and dermal effects of topical lactic acid* (Smith, 1996). By creating slightly acidic conditions (pH 4.5–5.5), lactic acid favors the growth of beneficial bacteria, which are more resilient than harmful bacteria due to stronger membranes and chemical defense systems (Khalfallah et al., 2021). Lactate, naturally present in human skin tissue, contributes to maintaining this acidic environment, known as the skin's acid mantle, which supports barrier function and regulates which microorganisms can grow on the surface (Petersen, 1999; Lamber et al., 2006). Many skin-associated bacteria are sensitive to pH changes, and more acidic conditions inhibit pathogenic growth (Lamber et al., 2006). Furthermore, lactate plays a role in skin immune regulation and metabolism (Ruan et al., 2025), and although it is unclear whether probiotic-derived metabolites reach the skin from the gut in significant amounts, lactate remains biologically relevant to skin health (Ruan et al., 2025). As a result, lactic acid may suppress the growth of *S. epidermidis* on the skin while still allowing beneficial bacteria to grow.

Staphylococcus Epidermidis

Staphylococcus epidermidis, or *S. epidermidis*, is a gram positive spherical bacteria that forms grape-like clusters (Lee & Anjum, 2023). While it is harmless in its natural habitat, an imbalance of *S. epidermidis* can lead to the increase in diseases in the human body (Claudel et al., 2019). Past researchers believed that *C. acnes* was the sole bacteria in the aid of forming acne. However, newer studies discovered that the balance and imbalance between *C. acnes* and *S. epidermidis* may be the cause of acne formation on the



human skin (Claudel et al., 2019). Research has not identified a specific ratio between these species for healthy skin, but rather the change in the interactions between *C. acnes* and *S. epidermidis* when the skin microbiome is disrupted (Eckhart & Sancar, 2020).

The Gut-Skin Axis

There is significant evidence that suggests that there is a connection between the skin and the gut. The gut-skin axis refers to the two-way relationship between the digestive system and the skin, influenced by immune responses, systemic inflammation, and changes in gut bacteria (Singla et al., 2025). The main mechanism that the gut participates in is the inflammatory immune response (Sánchez-Pellicer et al., 2022). mTOR, a nutrient regulator that aids in cell growth and the strengthening of the skin barrier, affects how the body handles diseases located on the human skin. Studies have shown that the types of food an individual takes can interact with the mTOR pathway (Sánchez-Pellicer et al., 2022). If an individual has a diet containing high glycemic loads, it may affect mTOR and lead to the increase of inflammation and irritate acne present (Sánchez-Pellicer et al., 2022).

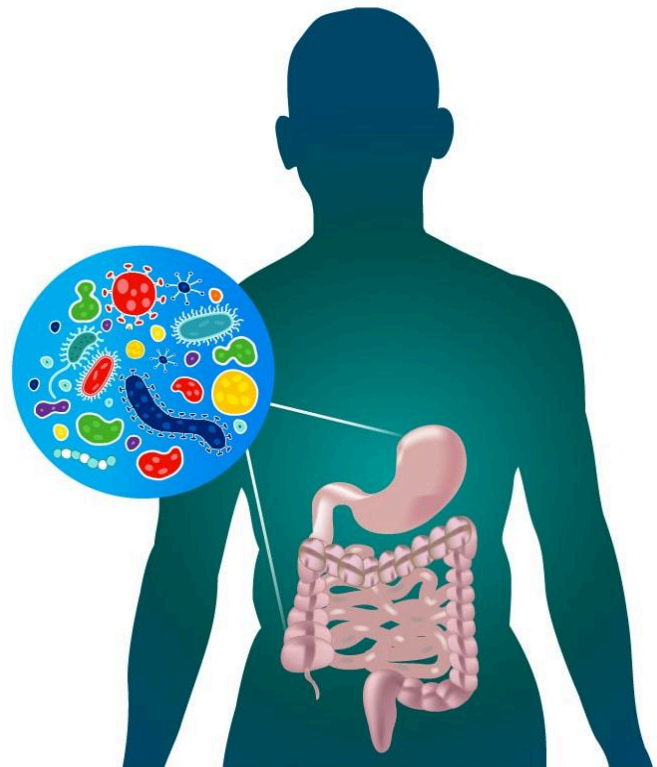
Leaky gut syndrome is a theoretical condition that is based on intestinal permeability (Cleveland Clinic, n.d.). A human gut is semi permeable, which allows nutrients to enter our bloodstream. However, some individuals have hyperpermeability, meaning that their bloodstream absorbs more than necessary nutrients (Cleveland Clinic, n.d.). This may allow for larger or toxic molecules to enter the bloodstream, triggering an inflammatory response (Cleveland Clinic, n.d.).

Previous Studies

There have been studies done on similar topics. Kober and Bowe, in 2015, found that probiotics can lead to an increase in the skin's hydration, as well as an improvement in the skin barrier function. They reported that retinoids, benzoyl peroxide, and other acne antibiotics have side effects on the skin, such as itchiness, redness, and dryness. Probiotics decrease these effects while keeping the skin healthy and strong.

The Gut Microbiome

The human gut microbiome is home to trillions of microorganisms, including but not limited to fungi, viruses, and bacteria that mainly reside in the large intestine. It plays an important role in digestion, metabolism, immune system development, and inflammation regulation. These microbes break down carbohydrates and fiber that the human body can not digest on its own, produce beneficial compounds (SCFAS - short chain fatty acids), and help produce vitamins like vitamin B and vitamin K (Cleveland



Clinic, 2023). The gut microbiome also strengthens the gut barrier. This prevents that absorption of harmful substances or toxins into the blood stream (Mann et al., 2020). Research shows that when the balance of gut bacteria present is disrupted (also known as dysbiosis), it can lead to an increase in inflammation and improper immune responses (Mann et al., 2020). For example, a

high in fat and low in fiber diet can lead to the alteration of gut composition and reduce diversity present, which can trigger inflammation among the body and lead to weight gain (Mann et al., 2020). The guts also contain a large amount of immune cells. The beneficial bacteria helps train the immune system to distinguish between harmless and harmful bacteria (Cleveland Clinic, 2023).

Recent studies show a connection between inflammatory skin diseases (eczema, psoriasis, and rosacea) and the gut microbiome. Individuals that suffer from these diseases often have different gut bacteria composition, having an increase in harmful bacteria and a decrease in beneficial microbiomes (Mann et al., 2020). Dysbiosis can increase gut permeability, which allows toxins to spread throughout the body and affect other organs, including the skin (Cleveland Clinic, 2023). This imbalance may lead to more skin inflammation. Some researchers suggest that probiotics may help restore the microbial balance that is necessary for a healthy microbiome, which may improve skin health (Mann et al., 2020).

Introduction

In this study, I tested whether metabolites produced by *Lactobacillus acidophilus* can reduce the growth of *Staphylococcus epidermidis*. To do this, I measured bacterial growth in two ways: OD600, which shows how cloudy the liquid culture is and indicates overall bacterial density, and CFU/mL, which counts the number of live bacterial colonies on plates. The treatment used tropical *L. acidophilus* metabolites, applied directly to the bacterial cultures. While it's not yet confirmed that these metabolites survive gut travel, there is some suspicion they could, making it interesting to test their effect directly. I hypothesized that the test groups receiving the metabolites would show less growth than the controls as the metabolites would inhibit the growth of *S. epidermidis*.

Variables

Table 1: Different types of Variables

Variable Type	What Is Included
Constant/Controlled	<ul style="list-style-type: none">- Volume of <i>S. epidermidis</i> (entire trial)- Total volume in test and control tubes (5 mL)- Incubation temperature and time period- Media used in growth and plating- Amount of light exposure- Dilution ratio for serial dilutions of the test and control tubes (1:10³ and 1:10⁶)
Independent/Manipulated	<ul style="list-style-type: none">- Type of treatment added to <i>S. epidermidis</i> (<i>Lactobacillus acidophilus</i> metabolites in LB broth or sterile LB broth)
Dependent/Responding	<ul style="list-style-type: none">- Growth of <i>S. epidermidis</i> (OD600 after 24 hour incubation and colonies on agar plate after 15 hour incubation)

Materials

Note: Proper PPE is required, as well as biohazardous disposal. All materials are sterilized in an autoclave beforehand.

Table 4: Different Materials Required

Biological Materials	Reagents and Media
<ul style="list-style-type: none">- <i>Lactobacillus acidophilus</i> capsules- Lyophilized <i>Staphylococcus epidermidis</i><ul style="list-style-type: none">- Innovating Science	<ul style="list-style-type: none">- Sterile distilled water- 70% ethanol- LB broth- Agar plates
Glassware & Plasticware	Equipment
<ul style="list-style-type: none">- 50 mL falcon tubes- 15 mL falcon tubes- Cuvettes- Micropipette tips- Sterile spreaders- Parafilm	<ul style="list-style-type: none">- Incubator (shaking and non-shaking)- Spectrophotometer- Centrifuge- Scale- Micropipettes (0.5-10 uL, 20 - 200 uL, 100 - 1000 uL, 1 - 5 mL)- Lab book- Pen

Procedure

Note: All plates and LB broth were prepared in advance using standard microbiology procedures, as well as the sterilization of all the materials used under a flow cabinet. This project is also a multi-day experiment, so plan accordingly.

Proof of Concept Procedure

1. Open a *Lactobacillus acidophilus* capsule and dissolve the powder in 5 mL sterile water.
2. Add the mixture to the prepared broth and incubate at 37°C for 24–48 hours to grow the bacteria.
3. Centrifuge the culture at 8,000 RPM for 15 minutes, then collect the supernatant (liquid)

using a sterile syringe.

4. In a separate flask, grow *E. coli* in broth at 37°C for 18–24 hours.
5. Dilute the *S. epidermidis* culture 1:10 — mix 1 mL of culture with 9 mL of sterile broth.
6. Prepare four sterile test tubes: label two “A” (control) and two “B” (test).
7. For tubes A (control): add 1 mL diluted *E. coli* + 1 mL sterile water.
8. For tubes B (test): add 1 mL diluted *E. coli* + 1 mL *Lactobacillus* supernatant.
9. Incubate all tubes at 37°C for 1–2 hours.
10. Measure bacterial growth at 600 nm (OD600) using a spectrophotometer with 1:4 dilution (750 μ L bacteria and 2.250 mL of Luria Broth)
11. Plate 100 μ L from each tube on nutrient agar and label clearly.
12. Incubate plates at 37°C for 24–48 hours.
13. Count bacterial colonies on each plate.
14. Compare colony numbers between control and treated plates and OD600 between control and treated plates
15. Repeat the experiment two times for accuracy.

Trial 1 Procedure

16. Open a *Lactobacillus acidophilus* capsule and dissolve the powder in 5 mL sterile water.
17. Add the mixture to the prepared broth and incubate at 37°C for 24–48 hours to grow the bacteria.
18. Centrifuge the culture at 8,000 RPM for 15 minutes, then collect the supernatant (liquid) using a sterile syringe.
19. In a separate flask, grow *Staphylococcus epidermidis* in broth at 37°C for 18–24 hours.

20. Dilute the *S. epidermidis* culture to 0.4
21. Prepare four sterile test tubes: label two “A” (control) and two “B” (test).
22. For tubes A (control): add 1 mL diluted *S. epidermidis* + 1 mL LB
23. For tubes B (test): add 1 mL diluted *S. epidermidis* + 1 mL *Lactobacillus* supernatant.
24. Incubate all tubes at 37°C for 24 hours.
25. Measure bacterial growth at 600 nm (OD600) using a spectrophotometer with 1:4 dilution (500 ul bacteria and 1.5 mL of Luria Broth)
26. Plate 100 µL from each tube on nutrient agar and label clearly.
27. Incubate plates at 37°C for 24–48 hours.
28. Count bacterial colonies on each plate.
29. Compare colony numbers between control and treated plates and OD600 between control and treated plates
30. Repeat the experiment two times for accuracy.

Trial 2 and 3 Procedure

Preparation of Bacteria

1. Open a *Lactobacillus acidophilus* capsule and dissolve the powder in 5 mL sterile water in a 50 mL falcon tube. Add 5 mL LB broth into this mixture.
2. In another 50 mL falcon tube with 10 mL LB broth, inoculated with *S. epidermidis*.
 - a. If the culture is dehydrated, first rehydrate the bacteria using the proper protocol provided by the manufacturer. In this case, the protocol provided by Innovating Science.
3. Incubate both cultures at 37°C at 200 rpm for 24 hours.

4. After the incubation period, plate 50 uL of each culture onto agar plates using a sterile spreader (one plate per culture) in order to maintain it for future use. Restreak every 1 - 2 weeks.
5. When ready to start trial, inoculate one colony from the plate of each bacteria strain into 10 mL of LB broth and incubate at 37° C at 200 rpm for 24 hours in sterile 50 mL falcon tubes.

Day 1: Trial Set Up

1. Check the OD600 using the spectrophotometer using the following steps.
 - a. Add 2 mL of fresh LB into a cuvette and blank the spectrophotometer.
 - b. Measure 500 uL of *L. acidophilus* into a cuvette and add 1.5 mL of LB broth.
(Creates a 1:4 dilution). As spectrophotometers are not accurate above an OD600 of 1.0, this will help to ensure appropriate readings (Wang et al, 2024).
 - c. Place the *L. acidophilus* cuvette into the spectrophotometer and measure the optical density of it. Multiply the reading by 4 to get the OD600 of the original culture.
 - d. Record the OD600 in the labbook
 - e. Repeat the same steps with *S. epidermidis*.
2. Centrifuge the *L. acidophilus* culture at 8 000 rpm for 5 min, then take the OD600 of the supernatant using the spectrophotometer and the steps above.
3. Prepare four sterile 50 mL falcon tubes: label C1 and C2 (for control group) and T1 and T2 (for test group).

4. For tubes C: Add 3 mL LB broth and 2 mL *S. epidermidis*.
5. For tubes T: Add 2 mL LB broth, 1 mL *L. acidophilus* supernatant, and 2 mL *S. epidermidis*.
6. Incubate all tubes at 37°C at 250 rpm for 24 hours.

Day 2: Serial dilutions and plating

1. Take the OD600 of all the trial tubes (c1, c2, t1, t2) using the spectrophotometer.
2. Prepare 2 sterile 15 mL tubes for each tube (Total of 8). Label them as follows: C1 1:10³, C1 1: 10⁶, C2 1: 10³, etc.
3. Add 4.955 mL of LB broth to each tube.
4. Add 5 uL of C1 to C1 1:10³ tube, using a pipette to thoroughly mix it.
5. Remove 5 uL of C1 1:10³ and add it to C1 1: 10⁶. Mix it thoroughly and expel 5 uL of C1 1: 10⁶ to keep the same volume.
6. Repeat steps 5 and 6 for the other 3 tubes.
7. Label the plates with the tube and the dilution factor: C1 1:10³, C1 1: 10⁶, etc. One plate for the 10³ dilution and two plates for the 10⁶ dilution (3 plates total for each starting tube).
8. From the appropriate tube, pipette 50 uL onto each plate and spread until dry with a sterile spreader.
9. Wrap and incubate the plates at 37°C for 24 hours.

Day 3: Counting of colonies

1. Count the bacterial colonies on the most countable plate (1:10⁶) for each tube.

2. Calculate the CFU/mL in the original culture.
3. Compare colony numbers between control and test group.

Repeat the experiment multiple times for greater accuracy.

Data

Table 3: OD600 of 24 hour inoculation cultures prepared for trial setup

Bacteria	OD600 (Trial 1)	OD600 (Trial 2)	OD600 (Trial 3)
<i>L. acidophilus</i>	0.692	0.520	0.531
<i>L. acidophilus</i> supernatant	0.296	0.180	0.130
<i>S. epidermidis</i>	0.196	1.000	0.986

Optical density at 600nm of cultures grown overnight in luria broth at 37C and 250rpm. A 1:4 dilution was performed using 1.5mL of broth and 500ul of culture before readings were taken on the spectrophotometer. Data represents the OD600 of the original culture.

Table 4: OD600 of Test and Control Tubes after Incubation

Tube Type	OD600 (Trial 1)	OD600 (Trial 2)	OD600 (Trial 3)
Control 1	3.068	2.616	3.704
Control 2	3.188	2.600	3.780
Test 1	2.720	2.560	3.304
Test 2	2.848	3.348	3.211

Optical density at 600 nm of control and test tubes at 37C and 250 rpm. Each test tube contained 2 mL of *S. epidermidis*, 1 ml *L. acidophilus* metabolites, and 2 mL luria broth while each control tube contained 2 mL of *S. epidermidis* and 3 mL of luria broth. A 1:4 dilution was performed

using 1.5 mL of luria broth and 500 uL of test/control tube. Data represents the OD600 of original cultures.

Table 5: CFU/mL for each Tube

Tube	CFU/mL (Trial 1)	CFU/mL (Trial 2)	CFU/mL (Trial 3)
Control 1	1.21 x 10 ⁹	6.50 x 10 ⁸	1.26 x 10 ¹⁰
Control 2	1.64 x 10 ⁹	7.29 x 10 ⁸	1.15 x 10 ¹⁰
Test 1	6.74 x 10 ⁸	1.20 x 10 ⁹	1.08 x 10 ¹⁰
Test 2	7.54 x 10 ⁸	1.10 x 10 ⁹	8.40 x 10 ⁹

Colony forming units per milliliter of cultures at 37C. Test and control tubes were serially diluted and plated on luria-bertani agar. For plates with high colony amounts (Trial 3), quadrant counting was used and values were multiplied by 4. In Trials 2 and 3, 2 plates were used per last serial dilution and averaged. CFU/mL values represent the bacterial concentration after all calculations.

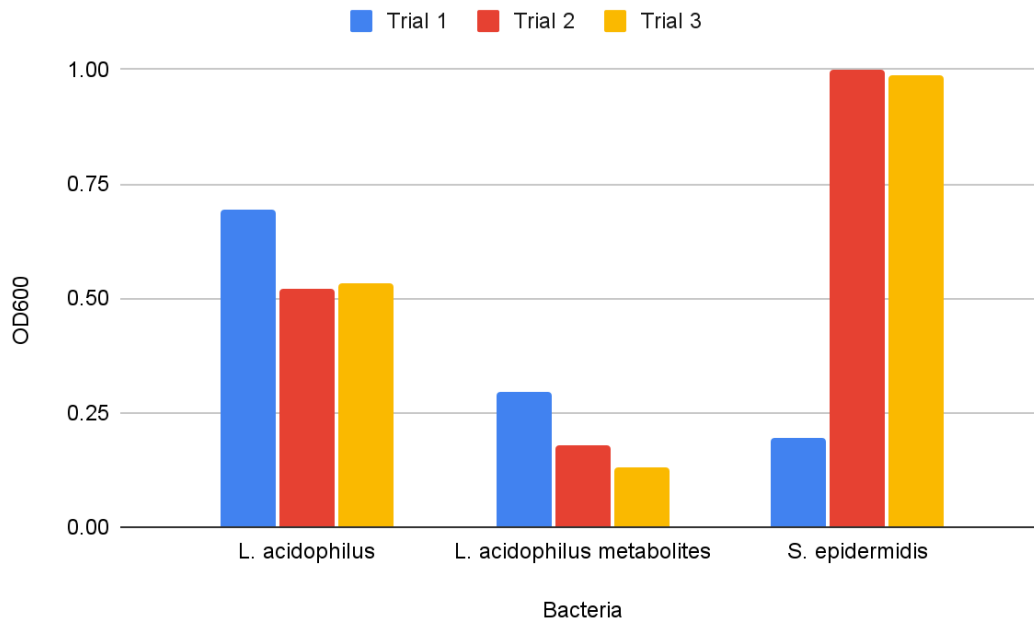


Figure 1. Initial OD600 of 24-hour inoculation cultures used for trial set-up. A 1:4 dilution was performed using 1.5mL of broth and 500ul of culture before readings were taken on the spectrophotometer. Data represents the OD600 of the original culture.

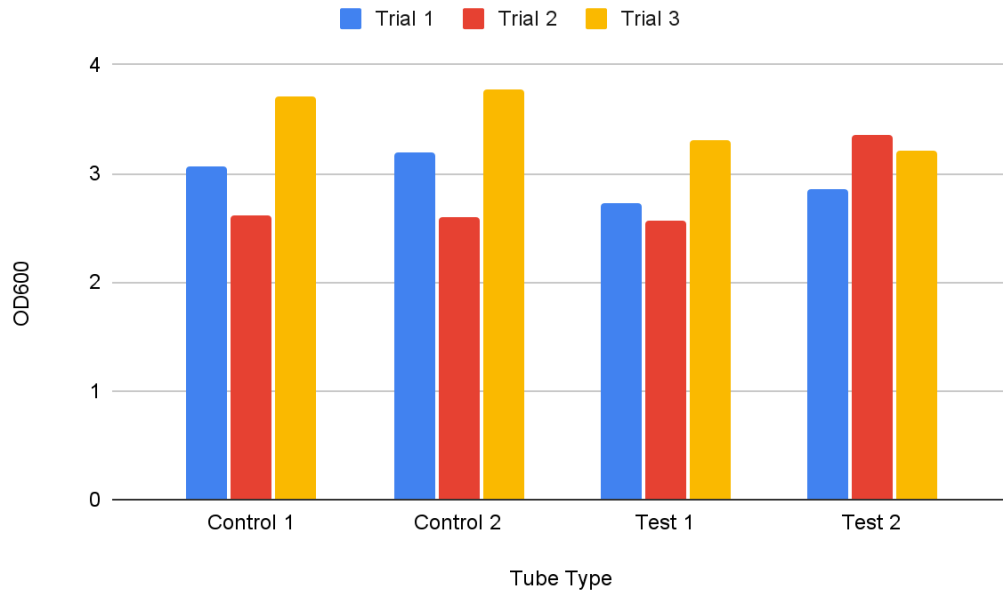


Figure 2. Final OD600 readings of control and test tubes following incubations at 37C and 250 rpm. Tubes were prepared using a 1:4 dilution (1.5 mL LB and 500 uL of culture) prior to the readings. Data represents the OD600 of original cultures.

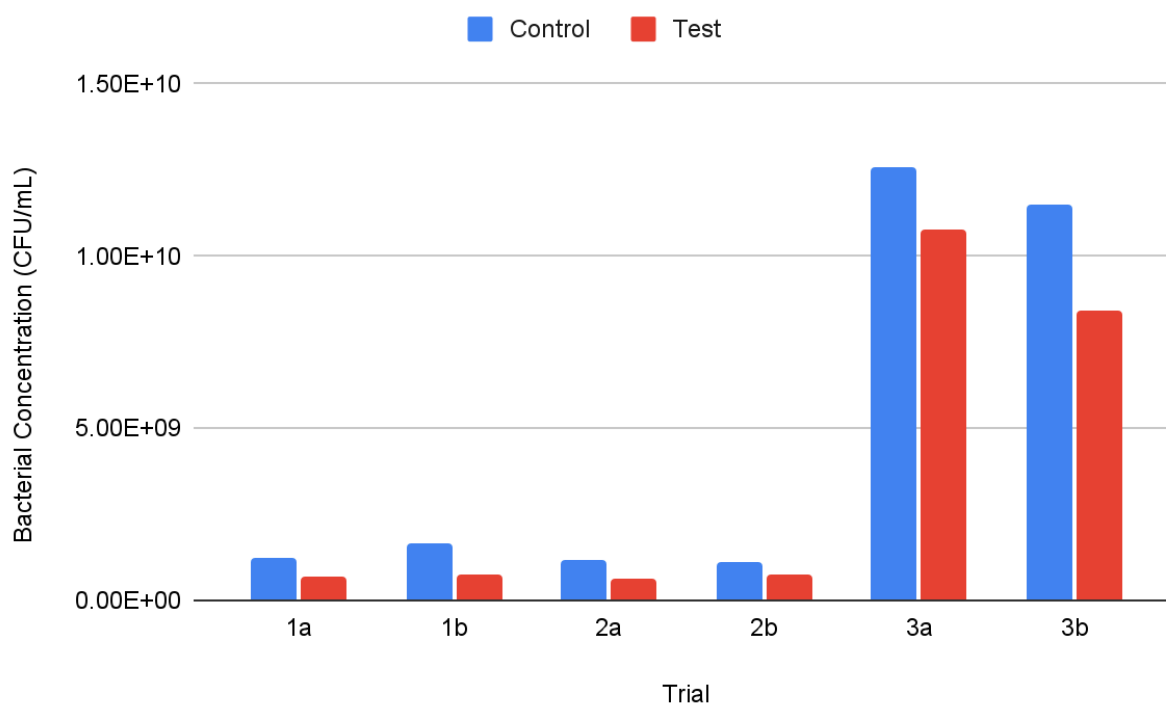


Figure 3. Colony-forming units per milliliter of control and test tubes after incubation at 37C. Samples were serially diluted and plated on LB agar. In Trials 2 and 3, 2 plates from the final serial dilutions were averaged. For Trial 3, quadrant counting was used for over populated plates and values were multiplied by 4. Each individual tube was accounted for (a and b in each trial). CFU/mL values represent bacterial concentrations after all calculations were made.

Table 6: Changes in Bacterial Growth (OD600) in Control and Test Tubes

Trial Number	Starting OD600	Finished OD600 Control	% Change in OD600 (Control)	Finished OD600 Test	% Change in OD600 (Test)
1a	0.0784	3.068	3813	2.72	3369
1b	0.0784	3.188	3966	2.848	3533
2a	0.4	2.65	540	2.616	554
2b	0.4	3.348	737	2.6	550

3a	0.394	3.704	840	3.304	739
3b	0.394	3.78	859	3.211	715

Optical density at 600 nm of control and test tubes was measured after incubation at 37C and 250 rpm. A 1:4 dilution was performed using 1.5 mL Luria broth and 500 uL of test or control tube. Each trial contained two control and two test tubes, and percent change was calculated for each tube (hence a and b for each trial). Data represents growth changes across all trials.

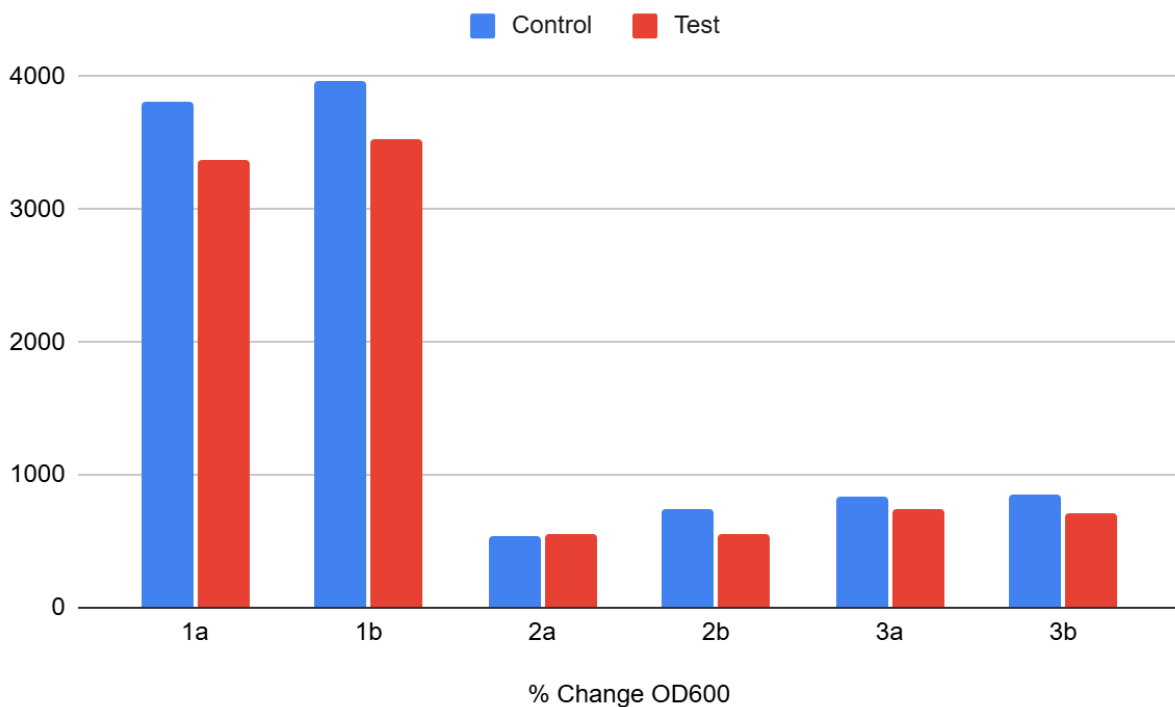


Figure 4. Percent Change in OD600 Over All Trials after incubation at 37c and 250 rpm.

Samples were diluted 1:4 using 1.5 mL of Luria broth and 500 uL of the test or control tube.

Each trial contained two control and two test tubes, and percent changes in OD600 were calculated for each tube (a and b in each trial). Data represent bacterial growth changes across all trials.

P value (CFU/mL): 0.038 & P value (OD600): 0.0354 (Results are significant)

Observations

The antimicrobial effect of *L. acidophilus* metabolite on *S. epidermidis* was measured through OD600 and CFU/mL. After incubation, the control tubes showed more bacterial growth than in the test tubes containing *L. acidophilus* metabolites. The control tubes had OD600 values of 3.188 and 3.068, while the test tubes had less growth, with values at 2.720 and 2.848. Additionally, after the growth had been spread onto agar plate and incubated overnight, the control tubes had higher colony counts with 1.21×10^9 CFU/mL and 1.64×10^9 CFU/mL. The test tubes had less growth with 6.74×10^8 CFU/mL and 7.54×10^8 CFU/mL. In trial 2, the results were not consistent. The OD600 values were similar to each other. The control tubes had OD600 values of 2.616 and 2.6000, while the test tubes had variation in the values at 2.560 and 3.348. CFU/mL had different values, with control tubes containing 6.50×10^8 CFU/mL and 7.29×10^8 CFU/mL, while test tubes contained higher growth values of 1.20×10^9 CFU/mL and 1.10×10^9 CFU/mL. In trial 3, the control tubes had OD600 values of 3.704 and 3.780, and test tubes had OD600 values of 3.304 and 3.211. CFU/mL for control tubes were 1.26×10^{10} and 1.15×10^{10} , while test tubes were slightly lower at 1.08×10^{10} and 8.40×10^9 .

Discussion

There were a few limitations and possible errors that might have affected my results. First, only a small number of trials were, so the results might not be super reliable. The experiment setup could have also caused some differences: test tubes had 2 mL of LB broth, while controls had 3 mL. Even though the total volume was still 5 mL, this probably gave the controls a tiny nutrient advantage. Plus, the 1 mL of supernatant in the test tubes came from an overnight *L. acidophilus* culture, which had already used up some nutrients. That means the test groups might have had less food for the *S. epidermidis*, which could have affected their growth. Other things that might have caused variation include pipetting mistakes, small differences in incubation time or

temperature, and possible mislabeling in Trial 2, which might explain why that trial was a bit inconsistent. Also, since the experiment was done in liquid instead of on real skin, it doesn't completely mimic what happens on human skin. This pattern was clearly seen in Trials 1 and 3, where Trial 1 showed a decrease from 3.128 to 2.784 in OD600 and from 1.43×10^9 to 7.14×10^8 CFU/mL, and Trial 3 showed a decrease from 3.742 to 3.258 in OD600 and from 1.21×10^{10} to 9.60×10^9 CFU/mL in the test groups compared to the controls. This suggests that *Lactobacillus acidophilus* metabolites can slow down *S. epidermidis* growth. It may have to do with things like lactic acid and hydrogen peroxide making the environment more acidic and less friendly for bacteria (*Epidermal and dermal effects of topical lactic acid* (Smith, 1996)). Trial 2 was less consistent, so more trials and tighter controls would help make the results more solid. Overall, the data supports the hypothesis that *L. acidophilus* metabolites reduce *S. epidermidis* growth, but more research would be needed before saying this happens for sure in real life.

Protocol Refinement

After the bacteria arrived, trial one started. Initially, 1 mL *S. epidermidis* and 1 mL of LB broth was added into the control tubes while in the test tubes 1 mL of *S. epidermidis* was added with 1 mL of *L. acidophilus* metabolites. The centrifugation was done at 8000 rpm for 15 minutes. Serial dilutions were done by the power of 10 5 times(10, 100, 10^3 , etc.) and 100 μ L of the final dilution was plated on large agar plates (one plate per dilution, so a total of 20 plates). After the trial was over, some alterations to the procedure were made. Centrifugation time was reduced to 5 minutes at 8000 rpm to prevent excess damage to the bacteria. To minimize the chance of pipetting error, the dilutions were changed so that only 5 μ L of culture was added (10^3 and 10^6) to 4955 μ L of LB. When counting colonies from trial one on the $1:10^5$ dilution, many colonies (870 and 640) were observed. $1:10^6$ dilution would have given

countable plates that are more reliable. Therefore, in further trials, it was decided to do 10^3 and 10^6 dilution to give countable plates and reduce pipetting error. Plating volume was decreased to 50 μ L on smaller plates to increase accuracy when counting plates. For the trial tube set up, the control tubes were changed to 3 mL LB broth and 2 mL *S. epidermidis* and the test tubes were changed to 2 mL LB broth, 2 mL *S. epidermidis*, and 1 mL of *L. acidophilus* metabolites in 50 mL tubes to allow for more oxygen. LB broth in all four tubes would allow for a more controlled environment to compare only metabolite effect, even though control tubes had one more millilitre due to limitations of this experiment. In the future, with resources and time, more trials should be done, as well as a bacterial growth curve. Additionally, a solution should be found to the same amount of resources to both test and control tubes to minimize differences.

Conclusion

The results of this experiment support the hypothesis that *L. acidophilus* metabolites reduce the growth of *S. epidermidis*. In trial one, the control tubes had approximately 50 % more bacterial growth than the test tubes. In trial two, the plates were very likely to have been switched, and trial three supports that claim. The trend of reduced bacterial growth with *L. acidophilus* metabolites was observed in trials one and three, and most likely trial two as well, with the test tubes showing less growth compared to the control tubes. This decrease in bacterial growth is likely due to the antimicrobial effects of *L. acidophilus* metabolites (lactic acid and hydrogen peroxide), that create an acidic environment and disrupt bacterial growth. Further trials and standardizing LB concentrations would enable more accurate results and decrease likelihood of errors.

Application and Future Research

These findings suggest that probiotic metabolites may have the potential to be used as an alternative to control skin bacteria that is related to acne while still keeping microbiome balance. Probiotics could be encouraged in skin care products to help reduce breakouts on teenage and adult skin. Future studies could also include additional trials, testing against other acne related bacteria, and finding out if different concentrations of probiotics deters bacterial growth. Additional trials can also allow for statistical analysis of the data.

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Glossary

Dysbiosis - When the microbiome is unhealthy. This is either when there is a loss of beneficial bacteria, increase in harmful bacteria, or lost balance of bacteria overall (Cleveland Clinic, 2023).

Gram Positive Bacteria - Bacteria that do not have a protective out wall. They have a thick cell wall and stain blue or purple on a gram stain test. They tend to be spherical or rod-like in shape, but can have branching filaments (Chen & Rowden, 2025).

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