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RESEARCH ARTICLE
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CONTROL OF ACNE ASSOCIATED MICROBES USING LACTIC ACID BACTERIA ISOLATED FROM COCKROACH GUT

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Abstract
Acne vulgaris, a widespread skin condition that effect almost 9.4% of the global population, primarily emerges in individuals aged fifteen to seventeen, with 15-20% facing moderate to severe manifestations. The conventional approach to treatment, predominantly relies on antibiotics, raises concerns about the development of antibiotic resistance. This research has been focused on lactic acid bacteria (LAB), specifically isolated from cockroach guts, as potential candidates for acne management due to their renowned antimicrobial and anti-inflammatory properties. The morphological traits of bacteria isolated from acne lesions revealed a diverse spectrum of the coexistence of multiple bacterial species. Molecular and Biochemical analyses showed isolation of gram-positive bacteria i.e. Staphylococcus epidermidis strain DSM 1922, Staphylococcus cohnii Strain F, and Streptococcus pyogenes strain mgas 28386 present in the acne lesions of this study. Antibiotic resistance profiling of these bacteria indicated strain-specific susceptibilities. Staphylococcus cohnii Strain F exhibited notable susceptibility to Azithromycin (31mm) and Erythromycin (30mm), while Staphylococcus epidermidis strainDSM 1922 displayed significant resistance. Streptococcus pyogenes strain mgas28386 showed mixed susceptibility, underscoring the importance of understanding strain-specific profiles. This study evaluated the antimicrobial effects of Lactobacillus planetarium strain mgas28386 and Lactobacillus rhamnosus strain 5974 against acne-associated pathogens, revealing strong antibacterial activity of Lactobacillus planetarium strain CE56.8, particularly against S. cohnii Strain F and S. epidermidis strainDSM 1922. Lactobacillus rhamnosus strain 5974 demonstrated moderate efficacy against S. pyogenes strain mgas28386.

Key words: Lactic acid bacteria (LAB), Probiotics, Acne vulgaris, Cockroach, Azithromycin

Introduction
Acne is a common skin disorder that is characterized as illness of the pilosebaceous unit in the skin that is related with an oil (Suva *et al*., 2014). Seborrhea (excess grease), non-inflammatory lesions (open and closed comedones), inflammatory conditions (papules and pustules), and scarring in varying degrees are the clinical aspects of acne (Kumar *et al*., 2023). Nearly everyone between the ages of fifteen and seventeen has some degree of acne, and 15-20% of young people have moderate to severe acne (Lee, 2020). According to estimates, acne affects 9.4% of the world's population,

making it the eighth most common disease globally (Piccolo *et al*., 2020). Up to 50 million cases of acne are reported in the United States each year. According to epidemiological research, acne is most prevalent in postpubescent teenagers and affects boys more often than girls, especially when the condition is more severe (Alqahtani *et al*., 2021). The age between 12 and 24, 85 percent of the adults have at least mild acne. Atopic dermatitis (AD) also affects up to 3% of adults globally and 20% of babies and adolescents (Grafanaki *et al*., 2023).

Multifactorial in nature, acne vulgaris is a skin disease caused by genetics, environmental and hormonal fluctuations (Lynn *et al*., 2016). The colonization of the *Propionibacterium acnes* (*P. acnes*) on the skin is a significant element in the development of acne. *P. acnes* is a gram-positive, anaerobic bacteria that often infects human skin, especially on the face, chest, and back, which produce a lot of sebum. *P. acnes* contributes significantly to the development of acne through a number of methods (McLaughlin *et al*., 2019). The swelling, redness, and pustules that are typically associated with acne can be caused by *P. acnes,* which can also cause an inflammatory reaction in the skin (Greydanus *et al*., 2021). *P. acnes* can also develop comedones, or plugged pores by creating enzymes that digest sebum and other lipids found in the skin. *P. acnes* can also promote the overproduction of fibroblast and accumulation of collagen, which can both contribute to the development of acne scars (Majeed, 2022). Inflammation, change in pH of skin, and increased sebum production are some of the main causes (Wu and Sivamani, 2022).

P. acnes is well known to cause anxiety, unhappiness with one's looks, an overall poor quality of life, and inhibits a person's social engagement (Charles *et al*., 2012). Acne can have a detrimental impact on quality of life, self-esteem, and mood, as well as increase the likelihood of anxiety, despair, and suicidal thoughts (Bhate and Williams, 2014).

Although antibiotics are frequently used to treat acne, concerns have been raised about the resistance among bacteria that cause acne (Karadag *et al*., 2021). Mutation and the exchange of resistance genes across bacterial species, can lead to antibiotic resistance (Harbottle *et al*., 2006). Resistance to the routinely prescribed medications erythromycin and clindamycin while treating *P. acnes* is observed (Mendoza *et al*., 2013). As a result, other antibiotics like tetracyclines and macrolides are now often used (Ianiro *et al*., 2016). Due to rise in number of acne cases, researchers are taking an interest in probiotics as a new therapy (Ribeiro *et al*., 2020). Probiotics are described as "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" (Martín and Langella, 2019). They do not have carcinogenic effects, but have a protective impact and are safe to use (Smith, 2019). Probiotic products are mostly used to cleanse and care for skin with atopic dermatitis, acne-prone skin, skin with eczema or psoriasis, and after invasive procedures in the field of cosmetics or medicine, including acid exfoliation (Chilicka *et al*., 2022). Probiotic bacteria include strains of Lactobacillus, Bifidobacterium, Streptococcus, and Saccharomyces etc (Aleta *et al*., 2020). Due to their antibacterial action against a variety of pathogens and their anti-inflammatory qualities, lactic acid bacteria (LAB), is an essential probiotic, may be used as a viable alternative therapy for acne (Brandi *et al*., 2020). LAB are often regarded as healthy and well-tolerated and may be found in fermented foods, the microbiota of human and cockroach guts, and dairy products (Rowan-Nash *et al*., 2019). It can also combat the problem of antimicrobial resistance across the world in different diseases (Terreni *et al*., 2021). LAB have also been found to offer potential as an alternate therapy for illnesses that are resistant to antibiotics since they do not encourage the growth of antibiotic resistance (Sahu *et al*., 2021).

Insects of the family Blattidae and order Blattodea include cockroaches (Monti *et al*., 2022). Cockroaches carry antimicrobial peptides produced by bacterial strains in their guts that have been demonstrated to have action against a variety of harmful bacteria, such as MRSA and *E. coli* (Siddiqui *et al*., 2023). These peptides may be employed to create novel antibiotics and serve as a treatment for bacterial infections (Jubeh *et al*., 2020). Therefore, cockroaches could prove to be a reliable source of LAB.

A cockroach's gut is home to a diverse microbial population that is crucial to the digestive process as well as other metabolic functions (Wang *et al*., 2023). There are many different types of bacteria, fungi, and other microorganisms found in the cockroach stomach, indicating a high level of microbial

diversity (Siddiqui *et al*., 2023). Studies have revealed the presence of lactic acid bacteria (LAB) in the cockroach gut microbiota, including species like *Leuconostoc*, *Lactobacillus*, and *Pediococcus* (Guo *et al*., 2020). *P. acnes* and other pathogenic bacteria have been proven to be susceptible to these LAB's antibacterial activities. The cockroach gut has a high microbial variety, thus it's feasible that it might be a source of LAB for use in treating acne and other disorders (Hassan *et al*., 2020). However, more investigation is required to determine whether the cockroach gut microbiome can serve as a source of LAB and to create efficient procedures for isolating and using these LAB to benefit human health.

In this study, Probiotic Potential of LAB isolated from the gut of cockroach has been evaluated against acne associated microbes possibly due to production of organic acids and other antimicrobial substances. Overall, this study intends to offer fresh perspectives on the use of LAB as a viable substitute therapy for acne vulgaris. This research was conducted to find more efficient and long-lasting acne therapies using LAB isolated from the cockroach's gut as a possible health advantage.

Material and methods

Collection of Cockroach

Cockroaches were collected from various locations using appropriate trapping and handling techniques. After that, they were transported to the Zoology lab. Each cockroach's body was cleaned with an alcohol swab to remove contaminants before dissection to expose the gut. The gut tissue was extracted under sterile conditions and placed in a test tube with 9ml of peptone water, being a nutrient solution, it facilitated the growth of microorganisms present in the gut sample.

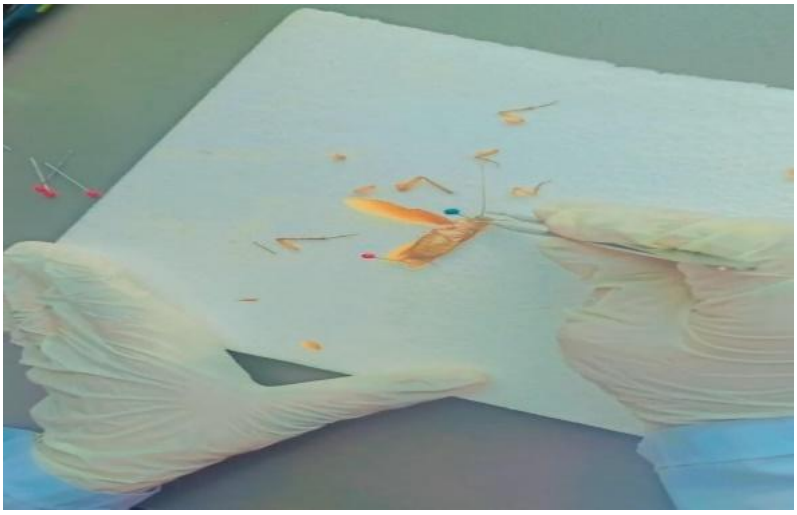

Figure 1. Cockroach Collection

Isolation of Lactic acid Bacteria

To obtain lactic acid bacteria, a 1% sample was added to 10 ml of MRS broth in a test tube, and then the mixture was incubated at 37ºC for 24 hours. After incubation, serial dilutions were made from the growth in the MRS broth. A volume of 0.1 ml from the last dilution was spread on the surface of MRS agar, which contains 1% $CaCO_3$. The petri dish was then incubated at 37ºC for 24 hours. After incubation, a colony surrounded by a clear zone was picked and transferred to another plate containing MRS agar for further purification. Colonies were purified by a round of streak and restreak method on MRS agar containing 1 % of $CaCO_3$. Pure colonies were stored in glycerol stock at -80C until further use.

Sample Collection for Acne

Firstly, the lesion was thoroughly cleaned with 70% ethanol. After that, it was pierced with a hypodermic needle to allow access to the affected tissue. To extract the sample, a comedone extractor was used to gently apply pressure to the lesion and collect the contents, which could have included

sebum, bacteria, and inflammatory cells. The extracted sample was then placed in a thioglycolate transport medium. To maintain the integrity of the sample, it was transported under cold conditions.

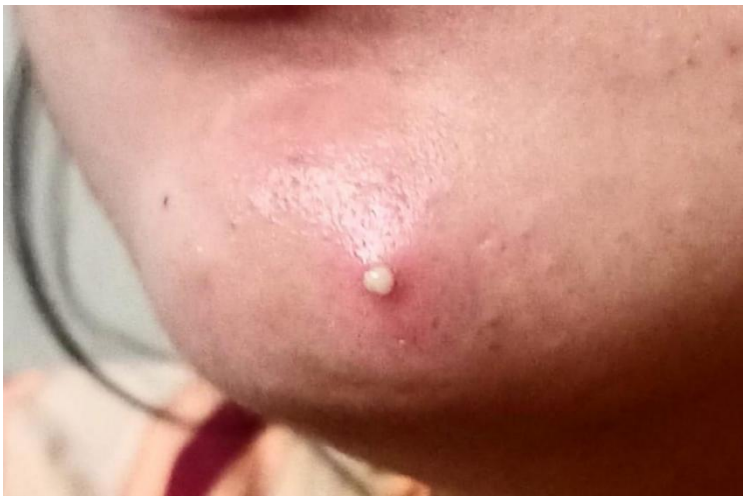

Figure 2. The sample collected from acne lesions

Figure 3. The sample transported through amies transport medium

Isolation of microorganisms from acne lesions

Each acne lesion sample was inoculated onto two plates of blood agar to promote bacterial growth. One of the plates was incubated aerobically for 48 hours at 37°C to promote the growth of aerobic bacteria, while the other plate was incubated anaerobically for 48 hours to promote the growth of anaerobic bacteria. The use of both aerobic and anaerobic cultures was important because acne lesions were known to harbor a diverse range of microorganisms, including both aerobic and anaerobic bacteria.

Figure 4. Anaerobic growth of acne associated bacteria

Biochemical Tests for Acne pathogen

To characterize the isolated bacteria from acne lesions, a series of biochemical tests were conducted. These tests aided in understanding various metabolic and enzymatic activities, contributing to the precise identification of bacterial species. The following biochemical tests were undertaken:

Gram Staining

The first step in identifying bacteria isolated from acne lesions involved Gram staining. This technique was employed to classify bacterial colonies as either gram-positive or gram-negative. The Gram staining process consisted of applying crystal violet stain, Gram's iodine, ethyl alcohol for decolorization, and safranin counterstain to the bacterial samples. The outcome of the Gram staining procedure provided essential information about the bacterial cell wall composition, thus narrowing down potential bacterial species.

Catalase Test

To determine the presence of the catalase enzyme, a small bacterial growth sample was mixed with hydrogen peroxide 34-36%. A positive result was indicated by the production of oxygen bubbles.

Coagulase Test

The coagulase test was performed in detail to assess the isolated bacteria's ability to produce coagulase enzymes. This involved emulsifying bacterial colonies in saline solution, adding plasma, and observing for immediate coagulation within a specified time frame.

Mannitol Fermentation Test

To evaluate mannitol fermentation capabilities, isolated bacteria were inoculated into mannitol salt agar. A change in the medium's color from red to yellow indicated mannitol fermentation.

Urease Test

The urease test was executed to determine if the bacteria possessed the urease enzyme. Bacterial colonies were inoculated onto urea agar, and the development of a pink or red color indicated urease activity.

PYR Test

The pyr test aimed to detect the presence of pyrrolidonyl arylamidase enzyme in the bacteria. Isolated colonies were tested with pyrrolidonyl-beta-naphthylamide reagent, and a positive result was indicated by a color change.

Oxidase Test
The oxidase test identified bacteria with the oxidase enzyme. A bacterial sample was applied to an oxidase reagent, and the appearance of a purple color within a specific time frame confirmed oxidase activity.

Motility Test
To determine the motility of the isolated bacteria, a sterile inoculation needle was used to inoculate bacterial cultures into SIM (Sulfide, Indole, Motility) medium. Motility was observed through the presence of outward radiating growth from the inoculation site.

Antibiotic resistance profiling of acne pathogen
To evaluate the antibiotic resistance profile of acne-causing pathogens, pure colonies of isolates were obtained and subjected to the disc diffusion method. Muller Hinton agar plates were swabbed with broth culture of bacterial strains, and commonly used antibiotics such as clindamycin, sulfamethoxazole, vancomycin, azithromycin, penicillin, and erythromycin were placed on the plates. The plates were then incubated at 37°C for 24 hours, and the diameter of the zone of inhibition was measured (mm) to determine the resistance and sensitivity of the isolates, as described by Moon *et al*. (2012). By performing these tests, we gained insight into the antibiotic resistance patterns of acne-causing pathogens, which can inform the development of effective treatment strategies.

16S rRNA sequencing based identification of acne associated pathogen
Pathogenic bacteria implicated in acne were isolated and identified through specific culture and biochemical tests as discussed above. Additionally, 16S rRNA sequencing were employed for precise identification. This integrated approach facilitates the comprehensive understanding and differentiation of LAB and acne-associated pathogens, crucial for elucidating their roles in acne pathogenesis.

Anti-Bacterial activity by Cell-Free Supernatant of LAB
To investigate the potential of LAB as a natural remedy for acne, we conducted a series of experiments were conducted to evaluate their effect on acne-causing pathogens.

Preparation of Cell Free Supernatant (CFS)
The process of producing CFS involved inoculating pure LAB strains in a suitable growth medium, such as MRS broth, which is a nutrient-rich medium commonly used for the cultivation of LAB. The inoculated broth was then incubated under optimal conditions for bacterial growth, typically at a temperature of 30°C for 48 hours under anaerobic conditions in incubator.
After the incubation period, the broth was subjected to centrifugation at a high speed of 10,000 rpm for 15 minutes. This caused the cells to sediment at the bottom of the centrifuge tube, while the liquid portion, or supernatant, was at the top. The supernatant contained all the soluble factors produced by the cells during growth, such as enzymes, peptides, and other bioactive compounds. In order to obtain a purified and sterile CFS, the supernatant was then filtered using a 0.2-micron membrane filter. This removed any remaining bacterial cells or debris, leaving behind a clear liquid that was rich in bioactive compounds produced by LAB.

Antibacterial activity of LAB
Once the cell-free supernatant had been filtered and collected, the antibacterial activity was assessed by subjecting the CFS to an antibacterial sensitivity test using the Kirby Bauer agar well diffusion method. A plate of Muller Hinton agar was inoculated with 10^6 CFU/ml of acne pathogenic strains. Sterile punctures were made in the agar to create wells, in each well 30ul of the cell-free supernatant of the LAB strain was poured. The plates were incubated for 24 hours at 37^0C, allowing the bacteria to grow and the CFS to diffuse out into the agar. After incubation, the plates were analyzed for zones of inhibition, which were clear areas around the wells where the bacteria had been killed or prevented

from growth due to the antibacterial activity of the CFS. The size of the zone of inhibition was measured (mm).

RESULTS

Morphological identification of bacteria isolated from acne lesions

The morphological traits of bacterial colonies isolated from acne lesions, revealing their diverse characteristics. These bacterial colonies showed various shapes such as round, irregular, or circular **Figure 5**.

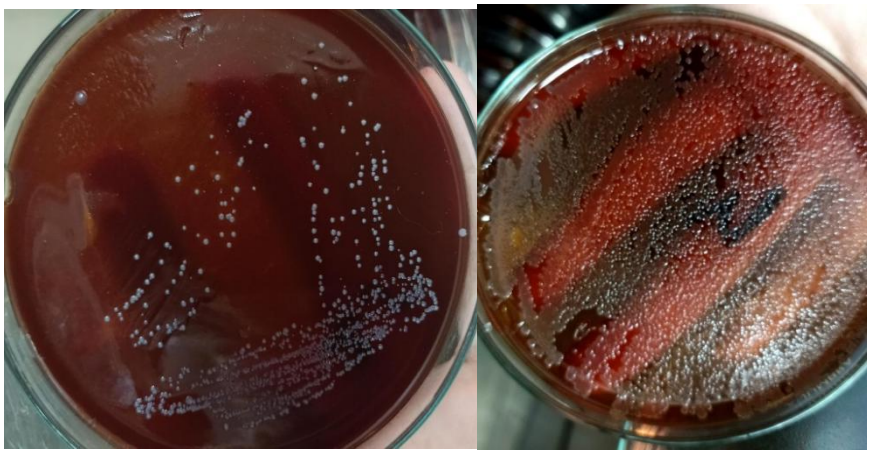

Figure 5. Different colonies of bacteria isolated from acne lesions

Biochemical tests for the identification of acne associated bacteria

The samples isolated from acne patients were subjected to various biochemical tests for the identification of acne associated bacteria. The results of Gram staining has showed that all of the samples are gram positive as shown in **Table 1**. Gram positive bacteria were subjected to catalase test and results indicated that all the samples are catalase positive except 2(s)1, 5L, E(6), Z(9), R(4), W(3), N(4) and P(2), thus it can be predicted that these catalase (+) bacteria are either Micrococcus species or it can be Staphylococcus species.

Table 1. Gram's staining and Catalase activity of acne associated bacteria

S.No.	Sample	Shape of bacteria	Gram staining	Catalase
1	5(2)S	Diplococci	+ve	+ve
2	W (3)	Cocci	+ve	-ve
3	7w(h)	Bacillus	+ve	+ve
4	N (4)	Cocci	+ve	-ve
5	17s	Cocci	+ve	+ve
6	2s(2)	Bacillus	+ve	+ve
7	2(s)1	Cocci	+ve	-ve
8	P(2)	Cocci	+ve	-ve
9	3s	Cocci	+ve	+ve
10	16s	Cocci	+ve	+ve
11	15(2)A	Cocci	+ve	+ve
12	E(6)	Cocci	+ve	-ve
13	2(s)l	Cocci	+ve	+ve
14	4S	Cocci	+ve	+ve
15	5O	Cocci	+ve	+ve
16	5L	Rod	+ve	-ve
17	Z(9)	Cocci	+ve	-ve
18	R(4)	Cocci	+ve	-ve

***+ve=positive, -ve=negative**

Mannitol fermentation and gas production test

These were performed by placing the Drihum tube for gas production to all catalase positive samples. The coagulase test was performed. Those samples which has shown yellow colour in mannitol fermentation test, coagulase-negative have been regarded either as *S. cohnii or Staphylococcus epidermidis.*

Thus the sample 17s have been considered as *S. cohnii.* The results of mannitol fermentation and Coagulase test have been illustrated in **Table 2**.

Table 2. Mannitol fermentation, Coagulase Test and Gas production test

S.No	Sample	Mannitol fermentation	Gas Production test	Coagulase Test
1	**5(2)S**	Red	+ve	+ve
2	**7w(H)**	Yellow	+ve	-ve
3	**17s**	Yellow	+ve	-ve
4	**2s(2)**	Red	+ve	-ve
5	**3s**	Red	+ve	-ve
6	**16s**	Yellow	+ve	-ve
7	**15(2)A**	Yellow	+ve	-ve
8	**2(s)1**	Light yellow	+ve	-ve
9	**4S**	Red	+ve	-ve
10	**5O**	Red	+ve	-ve

On the other hand, samples (2s(2), 3s. 4s and 5O) which has showed negative results for mannitol fermentation by maintaining the red colour and were Coagulase negative were further exposed to Urease test and Novobiocin sensitivity test.

Urease test and Novobiocin test

Urease test has shown that the two samples i.e 4s and 5O were negative by displaying a pure yellow colour while one sample 3s has displayed pure pink colour and sample 2s(2) has shown light pink. Therefore in order to further verify these two pink coloured samples for *Staphylococcus epidermidis,* a Novobiocin sensitivity test was performed which showed that 2s(2) sample have displayed zone of 15mm while 3s has shown 22mm. As 3s have shown a bigger zone as compared to 2s(2) thus it was regarded as pure *Staphylococcus epidermidis*

Table 3. Coagulase Test, Urease test, and Novobiocin sensitivity test

S.No.	Sample	Coagulase Test	Urease test	Novobiocin sensitivity
2	2s(2)	-ve	Light pink	18mm
3	3s	-ve	Pink	22mm
4	4s	-ve	Yellow	N/A
5	5O	-ve	Yellow	N/A

Identification of *S. pyogenes*

In order to identify *S. pyogenes* which is important acne causing pathogen, the gram negative, catalase-negative samples have been subjected for PYR test and hemolysis test as shown in Table 4. All those samples which were beta hemolysis were then tested for Bacitracin sensitivity test irrespective of the status of PYR test.

The sample N(4) was beta hemolysis and positive for PYR and shown a significant sensitivity zone i.e. 36mm have met the requirement of *S. pyogenes.*

Table 4. Hemolysis test, PYR, and Bacitracin sensitivity test

S.No	Sample	Hemolysis test	PYR	Bacitracin sensitivity test
1	**W (3)**	α	N/D	N/D
2	**N (4)**	β	+	36mm
3	**2(s)1**	γ	N/D	N/D
4	**P(2)**	β	-	N/D
5	**E(6)**	γ	N/D	N/D
6	**5L**	γ	N/D	N/D
7	**Z(9)**	α	N/D	N/D
8	**R(4)**	β	-	N/D
9	**7k**	B	+	19mm

16S DNA sequencing for identification of Acne associated isolates

In this molecular biology study, DNA extraction and subsequent analysis were conducted to identify bacterial species from three single colonies (3s, 17s and N4). The 16S RNA genes were purified using the WizPrep™ Gel/PCR Purification Mini Kit, and the purified samples were sent for sequencing. The obtained sequencing results were analyzed using BioEdit software as shown in Figure 6.

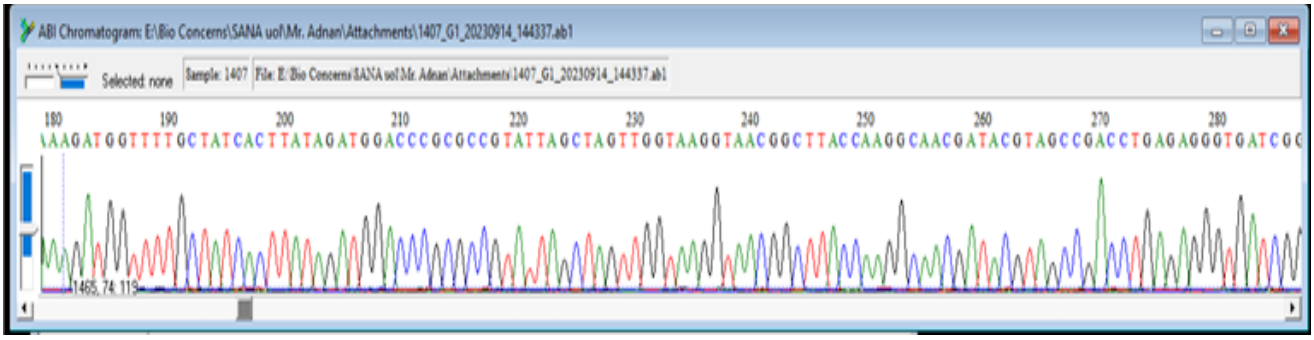

Figure 6. Analyzation using BioEdit software

The complete sequences were retrieved, and NCBI BLAST was employed for species identification. The identified species were *Staphylococcus epidermidis strain DSM 1922* from the sample 3s, *Staphylococcus cohnii Strain F* from the sample 17s, and *Streptococcus pyogenes strain mgas28386* from the sample N4 as shown in **Table 5.** This comprehensive molecular approach, combining DNA extraction, PCR, gel electrophoresis, sequencing, and bioinformatics analysis, allowed for accurate identification of bacterial species from the isolated colonies

Table 5. Identified species from the samples

Sample Name	Species
3s	Staphylococcus epidermidis strainDSM 1922
17s	Staphylococcus cohnii Strain F
N4	Streptococcus pyogenes strain mgas28386

Antibiotic resistance profile of acne associated isolates

The antibiotic resistance profiles of acne-associated bacterial strains are distinct. Starting with *S. cohnii*, the strain demonstrates notable susceptibility to most antibiotics, as reflected in relatively large zone diameters. Particularly noteworthy is the sizable zone of 31mm for Azithromycin (AZM), indicating high effectiveness against this strain. Erythromycin (E) also exhibits significant inhibition with a zone diameter of 30mm, suggesting effective management possibilities. Clindamycin (DA) and Vancomycin (VA) show moderate effectiveness, while Sulfamethoxazole (SMZ) and Penicillin (P) display moderate to low efficacy. In contrast, *S. epidermidis* showcases notable resistance to multiple antibiotics, notably lacking zones of inhibition against Erythromycin (E), Penicillin (P), and

Sulfamethoxazole (SMZ), indicative of high-level resistance. This aligns with the well-documented challenges associated with treating *S. epidermidis* infections due to its biofilm-forming ability and development of resistance. On the other hand, *S. pyogenes* presents a mixed resistance profile, demonstrating susceptibility to Erythromycin (E) and Penicillin (P), with significant zone diameters. However, reduced sensitivity is observed for Clindamycin (DA), Vancomycin (VA), Sulfamethoxazole (SMZ), and Azithromycin (AZM).

Table 6. Antibiotic resistance profile of acne associated isolates

S.No		**Antibiotics**					
1	**Strains**	**E(mm)**	**P(mm)**	**DA(mm)**	**VA(mm)**	**SMZ(mm)**	**AZM(mm)**
2	*S.cohnii*	30	14	10	15	16	31
3	*S.epidermidis*	N/A	N/A	14	9	N/A	N/A
4	*S.pyogens*	26.2	29.2	20.4	16.9	12.2	19.7

Table 7. Antibiotic resistance According to CLSI unit

S. No		**Antibiotics**					
1	**Strains**	**E**	**P**	**DA**	**VA**	**SMZ**	**AZM**
2	*S. cohnii*	S	R	R	S	S	S
3	*S.epidermidis*	R	R	I	I	R	R
4	*S. pyogens*	S	S	S	I	R	S

**** AZM= Azithromycin**
E= Erythromycin
P= Penicillin
SMZ= Sulfamethoxazole
DA= Clindamycin
VA= Vancomycin

Isolation and Identification of LAB

The lactic acid bacteria (LAB) identified with *Lactobacillus planetarium strain CE56.8* and *Lactobacillus rhamnosus strain 5974* previously isolated from the gut of cockroach, underwent a comprehensive examination through various biochemical tests, as detailed in the methodology section of this thesis. The results of these tests affirm that both selected strains exhibit characteristics consistent with LAB. Both strains are Gram-positive, indicating that their cell walls retain the violet stain, a typical trait of LAB. The absence of catalase, coagulase, and oxidase activities further supports their classification as LAB. In terms of morphology, strain L-1 appears as cocci (spherical cells) with a white color and transparency, while strain L-2 presents as rod-shaped cells with a yellow color and transparency. Both strains demonstrate growth on calcium carbonate, reinforcing their suitability for further investigation. These findings not only confirm the LAB nature of the strains but also provide crucial insights into their specific characteristics, laying the foundation for subsequent in-depth analyses and research outlined in the thesis.

Antimicrobial activity of LAB against Acne Pathogen

The study's results, which compared the susceptibility of acne-associated pathogen strains to two different LAB strains, *Lactobacillus planetarium strain CE56.8* and *Lactobacillus rhamnosus strain 5974*, revealed important insights into the potential of these LAB strains as probiotics for managing acne. In the context of *S. cohnii*, it was evident that *Lactobacillus*

Table 8. Comparison of the susceptibility of acne-associated pathogen strains

Sr #	**Gram staining**	**Catalase**	**coagulase**	**oxidase test**	**Shape**	**Color**	**Growth on Calcium carbonate**
L-1	Positive	negative	negative	negative	Cocci	white	Transparent
L-2	Positive	negative	negative	negative	Rod	yellow	Transparent

planetarium demonstrated a greater ability to inhibit its growth, as indicated by the larger zone of inhibition (31.3 mm), in comparison to *Lactobacillus rhamnosus* (27.9 mm). This finding implied that *Lactobacillus planetarium* might possess characteristics or produce metabolites that were more effective in countering the growth of *S. cohnii*, a bacterium associated with acne development.

A similar trend was observed when assessing the inhibition of *S. pyogenes*. *Lactobacillus planetarium* exhibited a larger zone of inhibition (29.5 mm) compared to *Lactobacillus rhamnosus* (25.2 mm). This suggested that *Lactobacillus planetarium* had the potential to be a more effective probiotic in combating S. *pyogenes*, another pathogen linked to acne.

However, the results took an interesting turn with *S. epidermidis*. In this case, *Lactobacillus rhamnosus* outperformed *Lactobacillus planetarium* significantly, displaying a notably larger zone of inhibition (33.2 mm) in contrast to the relatively smaller zone of inhibition exhibited by *Lactobacillus planetarium* (20.2 mm). This outcome suggested that *Lactobacillus rhamnosus* may have had specific properties or mechanisms that made it particularly effective in countering the growth of *S. epidermidis.*

These results highlighted the strain-specific nature of LAB effectiveness against acne-associated pathogens. The choice of LAB strain could significantly impact its ability to inhibit the growth of specific acne-related bacteria.

Table 9. Antimicrobial activity of LAB against Acne Pathogen

Acne associated pathogen strains	**LAB Strains**	
	Lactobacillus planetarium	*Lactobacillus rhamnosus*
S. cohnii	31.3mm	27.9mm
S. pyogenes	29.5mm	25.2mm
S. epidermidis	20.2 mm	33.2mm

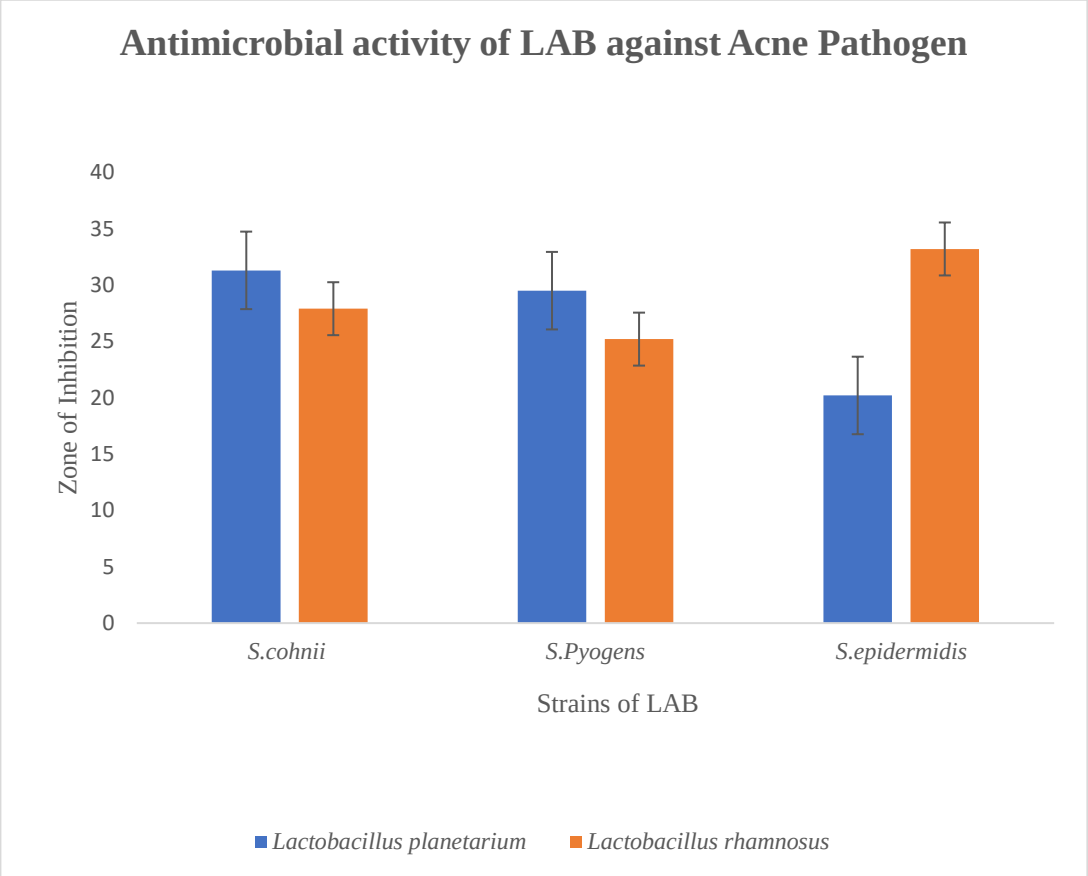

Figure 7. Graphical representation of antimicrobial activity of LAB against Acne Pathogen

Discussion

The escalating threat of global antimicrobial resistance (AMR) poses a significant risk to public health, as highlighted by alarming statistics (Majumder *et al*., 2020). The World Health Organization (WHO) reports that a minimum of 700,000 people succumb to drug-resistant infections annually, a number that could skyrocket to 10 million deaths per year by 2050 without prompt intervention (Abi-Ghaida, 2022). The crisis is increased by the overuse and misuse of antibiotics, fostering the development of resilient strains. This issue is due to insufficient development of new antimicrobial drugs in the pharmaceutical pipeline, exacerbating the problem (Sharma, 2020). A 2020 review commissioned by the UK government estimated that drug-resistant infections could incur a staggering cost of up to $100 trillion for the global economy by 2050. The consequences of the COVID-19 pandemic leads to the pandemic of antimicrobial resistance and there is crucial need to ensure the efficacy of essential medical interventions to secure the future of global healthcare (Adebisi, 2023).

With the global rise in antibiotic resistance, alternative approaches to treating various diseases are gaining attention, and probiotics are emerging as a promising option (Haque *et al*., 2022). Probiotics, which include bacteria like *Lactobacillus* and *Bifidobacterium*, as well as yeast like *Saccharomyces boulardii*, are live microorganisms known for providing health benefits (Ibrahim *et al*., 2023). They operate by maintaining gut microbial balance, modulating immune responses, and influencing metabolic processes including alleviating gastrointestinal issues, reducing infection risks, and supporting women's health (Solmi *et al*., 2023). Within the expansive realm of probiotics, Lactic Acid Bacteria (LAB) stands out as a diverse group of gram-positive bacteria crucial for their involvement in fermentative processes and their impact on various aspects of human health (Nandha and Shukla, 2023). Their primary function involves converting sugars into lactic acid through fermentation, a process integral to the preservation and flavor enhancement of fermented foods like yogurt, sauerkraut, and kimchi (Ibrahim *et al*., 2023). Beyond their role in food production, specific LAB strains, including *Lactobacillus* and *Bifidobacterium*, are acknowledged as probiotics, offering potential health benefits by positively influencing gut microbiota (Dahiya and Nigam, 2022). Lactic acid bacteria play a multifaceted role in human health, affecting diverse diseases. Notably, strains like *Lactobacillus* and *Bifidobacterium* significantly contribute to gastrointestinal health by maintaining a balanced microbiota, thereby alleviating conditions such as irritable bowel syndrome and inflammatory bowel diseases (Roy and Dhaneshwar, 2023). Their antimicrobial properties make LAB valuable in preventing and treating infectious diseases, spanning respiratory infections to urinary tract infections. Furthermore, LAB's immunomodulatory effects extend to metabolic disorders like obesity and type 2 diabetes, as well as mental health conditions, including anxiety and depression (Yadav *et al*., 2020). While promising, the strain-specific nature of LAB's effects emphasizes the ongoing need for research to optimize their use as probiotics in personalized disease management strategies. These bacteria contribute to maintaining a balanced microbial environment in the digestive system, promoting gastrointestinal health, and potentially enhancing immune function (Petruzziello *et al*., 2023). This study explores the potential antimicrobial properties of LAB against pathogens associated with acne lesions, considering the broader role of LAB in various microbial and non-microbial diseases and disorders

In this current study, distinctive antibiotic resistance patterns characterize the bacterial strains associated with acne. Starting with *S. cohnii strain F*, this strain exhibits significant susceptibility to a wide range of antibiotics, as evident from the notably large zone diameters. Notably, there is a large 31mm zone for Azithromycin (AZM), indicating high efficacy against this specific strain. Erythromycin (E) also proves effective with a substantial inhibition zone of 30mm, suggesting viable management options. Clindamycin (DA) and Vancomycin (VA) exhibit moderate effectiveness, while Sulfamethoxazole (SMZ) and Penicillin (P) show varying degrees of efficacy ranging from moderate to low. In contrast, *S. epidermidis strain DSM 1922* demonstrated significant resistance to multiple antibiotics, conspicuously lacking inhibition zones against Erythromycin (E), Penicillin (P), and Sulfamethoxazole (SMZ), indicating a high level of resistance. This result indicate that available antibiotics are not helpful in treating *S. epidermidis* infections due to its biofilm-forming ability and the development of resistance. Conversely, *S. pyogenes strain mgas28386* exhibits a mixed resistance

profile, being susceptible to Erythromycin (E) and Penicillin (P) with substantial zone diameters, while showing reduced sensitivity to Clindamycin (DA), Vancomycin (VA), Sulfamethoxazole (SMZ), and Azithromycin (AZM). A previous epidemiological study have shown diverse frequencies of antimicrobial resistance and susceptibility in different strains of acne-associated pathogens worldwide (Besednova *et al*., 2020).

In this investigation, it has been assessed the antimicrobial effectiveness of two distinct strains of Lactic Acid Bacteria (LAB) isolated from the cockroach gut, namely *Lactobacillus planetarium strain CE56.8* and *Lacticaseibacillus rhamnosus strain 5974*, against pathogenic strains associated with acne (*S. cohnii strain F, S. pyogenes strain mgas28386, and S. epidermidis strain DSM 1922*) based on the diameter of the inhibition zones. Notably, *Lactobacillus planetarium* demonstrated a substantial inhibitory impact on *S. cohnii*, as indicated by a 31.3mm zone diameter, signifying antibacterial activity. Conversely, *Lacticaseibacillus rhamnosus* exhibit 27.9mm zone of inhibition against *S. cohnii*, suggesting potential resistance or reduced effectiveness. Concerning *S. pyogenes*, both LAB strains showed inhibitory effects, with zone diameters of 29.5mm and 25.2mm for *Lactobacillus planetarium* and *Lacticaseibacillus rhamnosus*, respectively, implying a certain level of efficacy against *S. pyogenes*, with *Lactobacillus planterium* demonstrating slightly greater inhibition. Regarding *S. epidermidis*, *Lactobacillus planetarium* demonstrated significant antibacterial activity, reflected in a 20.2mm zone of inhibition, while *Lacticaseibacillus rhamnosus* exhibited inhibition of 33.2mm zone diameter.

The observed trends suggest that *Lacticaseibacillus rhamnosus* generally displayed a broader spectrum of antibacterial activity, particularly effective against *S. cohnii and S. epidermidis*, whereas *Lacticaseibacillus rhamnosus* exhibited moderate effectiveness, notably inhibiting *S. pyogenes*. Another study revealed the impact of *Lactobacillus fermentum TCUESC01* and *L. plantarum TCUESC02* supernatants, derived from the fermentation of fine cocoa, on the biofilm production of *Staphylococcus cohnii CCMB262*. The findings revealed that both Lactobacillus supernatants hindered the growth of *S. cohnii*, with *L. fermentum TCUESC01* notably reducing biofilm thickness, even at subinhibitory concentrations.

The findings reflects the need of progressive research to optimize LAB-based antimicrobial compounds for personalized disease management, offering a potentially effective and sustainable approach to acne treatment due to antibiotic resistance among bacteria.

Conclusion

Acne vulgaris, affecting a substantial percentage of the population, particularly adolescents worldwide. The intricate association with the pilosebaceous unit results in diverse clinical manifestations, ranging from non-inflammatory to inflammatory conditions, with potential scarring. The conventional use of antibiotics treatments leads to antibiotic resistance, prompting the exploration of alternative therapeutic strategies. Current study focus on lactic acid bacteria (LAB), specifically isolated from cockroach gut, presents a promising avenue for acne management due to their well-established antimicrobial properties. The morphological and molecular analyses of bacteria isolated from acne lesions reveal a diverse spectrum of gram-positive species, including *Staphylococcus epidermidis strain DSM 1922, Staphylococcus cohnii strain F*, and *Streptococcus pyogenes strain mgas28386.* Antibiotic resistance profiling underscores the importance of understanding strain-specific susceptibilities. The evaluation of *Lactobacillus planetarium strain CE56.8* and *Lacticaseibacillus rhamnosus strain 5974* antimicrobial compounds demonstrate their potential as an effective alternatives, particularly against *S. cohnii* and *S. epidermidis.* In addition, future research should broaden the exploration of LAB to identify additional strains with enhanced efficacy against acne-associated bacteria, thereby contributing to innovative and sustainable approaches for managing acne vulgaris.

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