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ANTIBIOFILM ACTIVITY OF PROBIOTIC LACTIC ACID BACTERIA FROM COCKROACH (PERIPLANETA AMERICANA) GUT

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Abstract
This study has been focused on Antibiofilm activity of probiotic lactic acid bacteria from cockroach (*Periplaneta americana*) gut. A total of 20 samples were collected from the cockroach gut isolating bacteria using a 10-fold serial dilution on agar plates incubated anaerobically. Further characterization of the isolated strains included assessing their morphology, Gram staining, and performing various biochemical tests. Isolated strains were evaluated for antimicrobial activity against indicators strains i.e., *E. coli, p. aeruginosa, s. aureus* and *klebsiella.* Wider zones of inhibition were observed in the case of LAB isolates against *E. coli*, with a range extending from 34mm to 20mm. Among 08 LAB strains 03 were resistant to streptomycin and vancomycin. Probiotic characterization included NaCl, pH, and bile salt tolerance, temperature resistance, auto-aggregation, co-aggregation, and cell surface hydrophobicity. Probiotic strains *Lactobacillus plantarum* CE56.8 and *Lacticaseibacillus rhamnosus* 5974 showed highest growth rate at 8% NaCl concentration. These strains displayed significant growth under various conditions, indicating their potential as probiotics. Importantly, these strains exhibited a notable ability to inhibit biofilm formation by *E. coli* (88%) and *P. aeruginosa* (67%), offer promising effect in combatting biofilm-related infections. explored promisingpossibilities in the domain of beneficial bacteria, including lactic acid bacteria isolatedfrom the gut of cockroach and lays the foundation for future investigations. However further research is needed to fully understand and develop LAB-based therapeutics forsuch infections.

Keywords: Cockroach, Lactic acid bacteria, Probiotic, Antibiotic resistance, Biofilm

Introduction

Bacterial resistance is a growing global concern as it refers to bacteria's ability to withstand antibiotics (Church and McKillip, 2021). Bacteria and fungi develop antimicrobial resistance due to self-medication (Dhasarathan et al., 2021). This leads to persistent growth and difficulty in treating infections. The presence of antibioticresistance results in higher mortality rates, prolonged hospital stays, and also increase healthcare costs. Several factors contribute to drug-resistance among bacteria, including overuse and misuse of antibiotics, serotype substitution, and mutations. According to Rosini et al. (2020), these bacteria can cause hurdle to treat infections. Another significant factor in antibiotic resistance is biofilm formation a structure formed by harmful bacteria. Bacteria in biofilms become resistant to various conditionsand substances, including antimicrobial agents, temperature changes,

and the body's defense mechanisms (Clutterbuck et al., 2007). In this scenario, probiotics are a potential solution. Research shows that beneficial bacteria can prevent and treat various illnesses (Sánchez et al., 2017). The idea of restoring a healthy microbiome has led to exploring new therapeutic approaches. Probiotics, including lactic acid bacteria, are known to be helpful (Akova et al., 2021). Using probiotics might reduce the growth andspread of drug-resistant bacteria. The body's natural microbiota, including bacteria, viruses, and fungi play vital role in fighting with pathogens. The microbiota, which exists in large numbers in the human body, helps combat infections by competing harmful bacteria, producing antibiotic substances, and maintaining a strong immune response (MedinaFélix et al., 2023). Combining probiotics with a balanced diet and lifestyle can lower the risk of bacterial resistance and improve overall well-being (Flandroy et al., 2018).

Probiotics are emerging viable microbes used as an alternative to chemotherapeutic agent used to established normal flora and preventing the occurrence of diseases. During infection, they can modulate the immune response, engaging both the innate and adaptive immune systems (Kanauchi et al., 2018). Notably, Lactobacillus spp. exhibit exceptional probiotic attributes. When probiotic microorganisms present in sufficient amount within the digestive system, they establish a balance equilibrium between harmful and beneficial gut microbiota (Yadav and Jha, 2019).

Probiotics positively influence host physiology by dietary supplementation withthese microbes by increasing immunity, improving nutrition, and encouraging microbial balance in the digestive tract (Reddy et al., 2011).

Lactic Acid Bacteria (LAB) showed probiotic potential and role in limiting theestablishment of hazardous organisms have opened up new opportunities in the domains of medical sciences and food biotechnology in recent decades (Raksasiri, 2016). These probiotic LAB strains have made significant contributions in fields of research including physiology, genetics and microbiology. Lactic acid bacteria are Gram-positive, acid-tolerant, non-spore former and can be rod-shaped (bacilli) or spherical (cocci) in shape (Kumar et al., 2022).

Several LAB strains contain proteinaceous bacteriocins that inhibit spoilage and harmful microbes. (Pandey et al., 2013). Lactic acid and other metabolites improve food quality and maintain texture. The use of LAB in the industry has increased due to its GRAS status, which is because of its widespread presence in food and role in maintaining balanced microflora on human and animal surfaces. (Bhogoju and Nahashon, 2022).

Biofilms are populations of bacteria that attach to surfaces and produce a protective framework known as extracellular polymeric substances (EPS) (Mahto et al., 2022). Biofilms can form on various surfaces, including medical devices, and are hard to eradicate with traditional antibiotics. LAB inhibit biofilm development by preventing bacterial adhesion, disrupting the EPS matrix, and entering established biofilms to inhibit growth. LAB produce organic acids, hydrogen peroxide, and bacteriocins, enhancing their effectiveness against biofilm-related illnesses (Giordani et al., 2021).

Biofilms offer a substantial danger in the food industry because they can cause pathogenic and spoilage microorganisms to contaminate food items. Biofilms grow onsurfaces and serve as long-term bacterial r eservoirs (Schulze et al., 2021). Furthermore,biofilms can serve as reservoirs for pathogens and spoilage bacteria and to survive from cleaning and disinfection treatments. In fact, biofilm-contaminated equipment played arole in 59% of foodborne disease outbreaks evaluated in France (Khelissa et al., 2017).

Biofilms are surface-attached microbial colonies that cause 75% of human microbial illnesses. They have been linked to otitis media, infective endocarditis, atherosclerosis, sialolithiases, bacterial vaginosis, and mastitis (Bhowmik et al., 2021).Sessile bacteria in biofilms resist stressors like anti-infective drugs. Some microbes with anti-biofilm properties inhibit growth, exclude competitors, and dominate in these competitive communities. (Miquel et al., 2016).

Biofilms play an important role in microorganism survival by supporting the growth, clustering, and maturation of various bacteria and fungi living together (Karygianni et al., 2020). Bacteriocins, unlike

antibiotics, target closely related or identical strains and are produced during primary growth phases through ribosomal peptide synthesis. (Westhoff et al., 2021). Bacteriocins are now frequently used as a natural alternative to synthetic antibiotics, which pose antibiotic resistance in humans and animals, due to their use in foods as biopreservatives and treatments for various disease (Tshibangu-Kabamba and Yamaoka, 2021).

Numerous studies have suggested that cockroach gut microbiota may be developed into effective antibiotic or probiotic for the treatment and prevention of drug-resistant pathogens, potentially offering a solution to the current major public health crisis (Siddiqui et al., 2023).

Cockroach species are associated with human habitats, and some are notorious pests. The insect stomach is important for digesting, food absorption, detoxification, and oxidative stress responses (Zhang et al., 2016). Cockroaches are highly adaptable, but their gut microbiome's potential remains unexplored. This study aims to investigate the therapeutic potential of this microbiome. Specifically, it will examine the antibiofilm effectiveness of lactic acid bacteria with probiotic properties from the American cockroach (*Periplaneta americana*) that may inhibit various infections.

Material and Methods

Sample collection

Cockroaches were collected from residential and commercial settings using trapping techniques and transported to the Zoology lab. Each cockroach's body was cleaned with an alcohol swab to remove contaminants before dissection to expose the gut. One gram of gut tissue was extracted under sterile conditions and placed in a test tube with 9 ml of peptone water, being a nutrient-rich solution, facilitated the growth of microorganismspresent in the gut sample. The sample was then mixed thoroughly to disperse microorganisms.

Figure 1. Cockroach gut in Zoology Lab

Serial Dilution & Isolation

A 10-fold serial dilution was prepared by mixing 1 ml of the sample with 9 ml of peptone water. For bacterial isolation, 1 ml of each dilution was spread on MRS agar supplemented with 0.3% CaCO3 and incubated anaerobically at 37°C for 24 hours. After incubation, distinct colonies were selected, purified through successive streaking, and stored in glycerol stock at -80°C. MRS agar plates enriched with 0.75–1% CaCO3 were used to identify lactic acid production, where colonies with hollow zones were considered positive for lactic acid.

Characterization of isolated strains

Biochemical test

Biochemical tests are essential for identifying bacterial strains and diagnosing infections by assessing their metabolic and enzymatic activities. Tests like oxidase, catalase, and coagulase identify specific enzymes, while urease, TSIA, and carbohydrate fermentation reveal metabolic pathways. Motility, Methyl Red, Voges-Proskauer, and hemolytic activity tests further distinguish bacteria based on movement, acid production, and red blood cell breakdown, aiding in precise bacterial classification.

Evaluation of the lactic acid bacteria's (LAB) antibacterial potential
The antibiotic activity of LAB strains was evaluated using the Agar well diffusion assay(AWDA)

Agar well diffusion assay (AWDA):
Using the Agar well diffusion experiment, the antibacterial activity of LAB strains against pathogens was assessed. In this procedure,four pathogens (indicators) were spread on the surface of the agar, including *E. coli, klebsiella, S. aureus, and Pseudomonas aeruginosa*. Under aseptic conditions, 6 to 8 mm diameter hole was punched with a sterile puncture and 100ul of the LAB strain supernatant was pour into the well, and incubate for 24 hours.

Antibiotic profile of LAB strains
The selected LAB strains were screened for sensitivity to eight clinically relevant antibiotics: tazobactam, erythromycin, vancomycin, clindamycin, ciprofloxacin, imipenem, streptomycin, and tetracycline, using the Kirby-Bauer Disc diffusion method. The exponentially growing LAB culture was spread on Muller Hinton agar plates (Table3.3). Place the antibiotic disc separately on the Muller Hinton agar plate and incubate at 37°C for 24 hours. By spreading LAB isolates on Muller Hinton agar, the antibiotic susceptibility of selected strains is screened. Place the disc on the plate and incubate the plate on 37°C for 24 hours. Zone of inhibition was measured in (mm) and designated as sensitive, resistant, and intermediate, followed by the (CLSI) standard.

Determination of probiotic properties of LAB Strains
The probiotic potential of LAB strains was assessed through NaCl, pH, bile salt, and temperature tolerance tests by measuring optical density after incubation. Each test evaluated the growth response of LAB under varying conditions of salt, acidity, bile salt concentration, and temperature.

Auto-aggregation
The strain was incubated for 18 hours at 37°C centrifuged for 4 minutes at 10,000 rpm. The cells were then rinsed twice with phosphate-buffered saline (PBS) and resuspended to a concentration of 10^8 cfu/mL. 4 mL of the probiotic strain solution was poured separately into sterile tubes and aggressively stirred in orderto test the ability to auto-aggregate. 150-µl aliquots of the top suspension were then removed from these test tubes at various time intervals (0, 4, 8, 18, and 24 hours) whilethey were kept at room temperature without being stirred. A spectrophotometer was used to measure the optical density (OD) at 600 nm . The following equation was used to determine the auto-aggregation percentage:

$$\% \text{ Auto-aggregate} = (A_0 - A_t) / A_0 \times 100$$

Where A_0 is the optical density at time 0, and A_t is the optical density at different timeintervals.

CO-aggregation
A sterile test tube was filled with 2 mL of probiotic strain and pathogen culture,and vortexed to assess the co-aggregation. At 0 and 4 hours, aliquots of 150 µL of the suspension were taken, and their absorbance at 600 nm was measured with a spectrophotometer in these tubes at room temperature without stirring, following the method by Pachla et al. (2021). The co-aggregation percentage was calculated asfollows:

$$[1\text{-}OD_{mix} / (OD_{strain} + OD_{pahogen})/2] \times 100.$$

Where $OD_{pathogen}$ and OD_{strain} are the absorbances of pathogens and LAB at 0 h, Respectively, and OD_{mix} is the absorbance of the mixed suspensions at 4 h.

Cell surface hydrophobicity
Shi et al. (2020) developed a modified approach for determining cell surface hydrophobicity. Overnight cultures of LAB strains were centrifuged for15 minutes at 10,000 rpm and 4°C.The cells

were resuspended in PBS buffer (Table3.12) after the pellet was washed twice with PBS buffer. The optical density of the cell suspension (OD 600nm) was measured and recorded as A_0. The cell suspension and xylene (a hydrocarbon) were then extensively combined by vertexing. After the mixture had formed two layers, it wasincubated at room temperature for 30 minutes. Following that, the aqueous phase was carefully removed, and its absorbance (OD 600nm) was measured again and recorded as A. The following formula was used to determine hydrophobicity:

$H\% = [(A_0 - A) / A_0] \times 100$

where A_0 and A represent the absorbances before and after mixing with xylene,respectively.

Antibiofilm activity of probiotics:

Antibiofilm activity was determined using a previously published method that was slightly modified. Fresh sterile tryptone soya broth (TSB)(Table3.4) was used to grow the pathogenic bacteria overnight. Then, 160µL of each bacterium's culture was transferred to 96-well microtiter plates. Except for the negative controls, 40 µL of LABbacterial supernatants were added to each well in order to achieved a final volume of 200 µL. The medium was discarded after 24 hours of incubation at 37 °C. Following that, the biofilms were fixed with 200 µL methanol for 10 minutes, stained with 150 µL0.1% crystal violet for 10 minutes, and gently rinsed three times with water (Nasr-Eldinet al., 2017). A microplate reader was used to measure the absorbance at 590 nm as thevalue of biofilm formation. The mean absorbance (OD595 nm) of test organisms was obtained, and the percentage inhibition was calculated using a specific formula.

Percentage inhibition = 100 – ((OD595nm test for positive control well/OD595nmnegative control well) ×100).

DNA Extraction

The chosen strain's broth culture underwent centrifugation at 10,000 rpm and 4°C. Following this, the supernatant was removed, and the pellet was reconstituted. Subsequently, 400 µL of TE buffer and 100 µL of 10% SDS were gently mixed, followed by the addition of 5 µL of Proteinase K. This mixture was then incubated at 37°C for 1 hour. Next, 500 µL of phenol was added, and the mixture was stirred at roomtemperature for 4-5 minutes. Afterward, it was centrifuged at 10,000 rpm for 10 minutes. The resulting aqueous supernatant was transferred to a fresh Eppendorf tube for the following steps, while the remaining material was discarded. To this supernatant,500 µL of either potassium acetate or sodium chloride at pH 5.2 was added, followed by 1 mL of isopropanol. The mixture was then frozen and subjected to centrifugation at 10,000 rpm for 10 minutes. The supernatant was discarded, and the remaining pelletwas retained for the next phase. A washing step with 70% ethanol was performed, andthe samples were subsequently centrifuged at 5,000 rpm for 5 minutes. Finally, 25 µL of injection water was added to each Eppendorf tube.

Gel-Electrophoresis

Prepare the TAE support solution by transferring 100 mL of buffer into a conicaljar. Measure 2 grams of agarose and add it to the 100 mL buffer solution. keep for a while in the oven. Take from the oven arrangements. Introduce bromide with ethidiuminto the mixture. For gel casting, pour the resulting solution into the mold and put the pepper. Then, pour the prepared gel solution into the electrophoretic chamber using the100 mL buffer. In the electrophoretic chamber, carefully position the gel within the caster. Connect the cathodes and activate the system. Following this, power down the supply after 45 to 50 minutes. Remove the gel from the electrophoretic chamber. Placethe gel under the UV transilluminator and turn on the transilluminator to examine the results.

16S DNA gene sequencing

To determine the genus of the isolated strain, we conducted DNA sequencing of the 16S ribosomal RNA gene. This sequencing process involved amplifying the specific gene of interest using universal primers designed for 16S ribosomal RNA genesequencing, which were obtained from:

27F (5'-AGAGTTTGATCCTGGCTCAG-3')

To

1392R (5'GGTTACCTTGTTACGACTT-3'

Results

This study was designed to investigate antibiofilm activity of probiotic lactic acid bacteria from cockroach (*Periplaneta americana*) gut.

Antagonistic activity

Agar well diffusion method

By using the agar well diffusion method against indicator organisms such as *E.Coli, Klebsiella, S. aureus,* and *P. aeruginosa*, the antagonistic potential of isolated strains was assessed. The strains that were examined exhibited varying degrees of antagonistic activity. Table 4.3 shows that 3 out of the 8 strains tested showedsignificant inhibitory effects against both Gram-positive and Gram-negative indicator strains. The LAB isolates showed wider zones of inhibition against *E. coli*, ranging from 34mm to 20mm (Figure 4.17). In contrast, testing against *Klebsiella* resulted in the lowest zones of inhibition, which ranged from 19mm to 12mm (Figure 4.15).

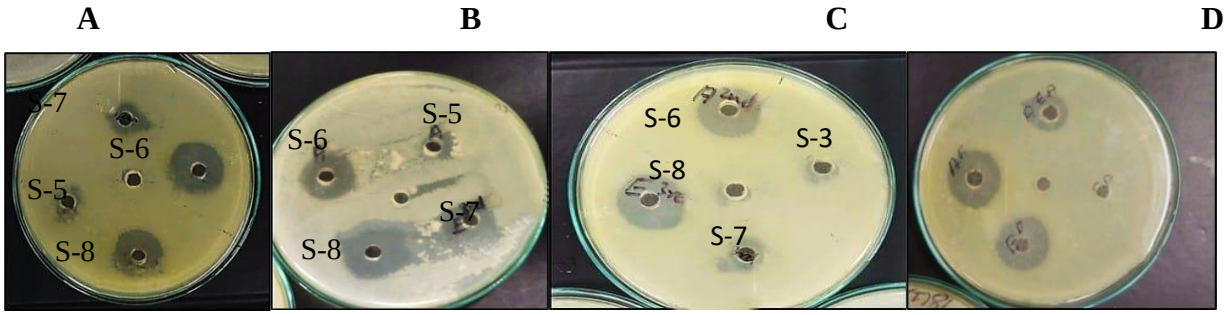

Figure: Antimicrobial potential of LAB isolates demonstrated through inhibition zones against key pathogenic bacteria: **(A)** *Pseudomonas aeruginosa*, **(B)** *Staphylococcus aureus*, **(C)** *Escherichia coli*, and **(D)** *Klebsiella* spp. The clear zones around the isolates indicate their effectiveness in combating these harmful microorganisms**.**

Table 1. Zone of inhibition of isolated strains against pathogens by AWDA

Strain	***E. coli*** **mm**	***Pseudomonas*** **mm**	***Klebsiella*** **mm**	***S. aureus*** **Mm**
S-1	20	-	12	-
S-2	24	19	9	15
S-3	17	21	-	11
S-4	24	-	10	-
S-5	-	20	-	16
S-6	31	29	11	27
S-7	29	30	19	30
S-8	34	32	12	33

mm= millimeter

Antibiotic profiling of LAB strains

Selected LAB strains showed different response against commonly usedantibiotics i.e., tazobactam, erythromycin, vancomycin, clindamycin, ciprofloxacin, imipenem, streptomycin, and tetracycline using Kirby Bauer disc diffusion method. Among 8 LAB strains 03 were resistant to streptomycin and vancomycin 01strain wasonly resistant to streptomycin while sensitive to all other antibiotics

Table 2. Antibiotic resistance activity of isolated LAB strains

Sr	Antibiotic disc	S-1 mm	S-2 mm	S-3 mm	S-4 mm	S-5 mm	S-6 mm	S-7 mm	S-8 mm
1	Tazobactam	S	S	S	S	S	S	S	S
2	Ciprofloxacin	S	S	S	S	I	I	S	R
3	Vancomycin	R	R	R	R	R	R	R	R
4	Clindamycin	S	S	R	R	S	S	S	S
5	Erythromycin	S	S	S	S	S	S	S	S
6	Imipenem	S	S	S	S	S	S	S	S
7	Tetracycline	S	I	I	R	S	S	S	S
8	Streptomycin	R	R	R	R	R	R	R	R

LAB strains = S-1, S-2, S-3. S-4, S-5, S-6, S-7, S-8

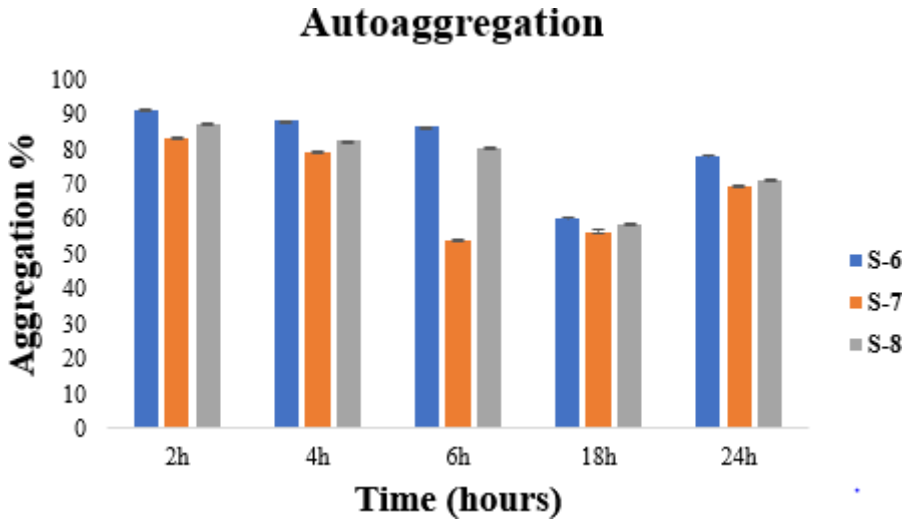

Figure 2. Comparison of auto-aggregation activity of LAB strains on differenttime intervals

One-way ANOVA (p<0.05) was used to evaluate the data, followed by the Tukeypost hoc test. The results are shown as Mean ± SEM.

CO-aggregation

Safety testing was performed using a coaggregation assay that was quick, repeatable, and simple to perform. The congregative qualities of two strains with strong auto aggregation capability were examined.co-aggregation was tested of three strains for three pathogenic bacteria (*p. aeruginosa*, *Klebsiella* and *S. aureus*). A considerabledegree of coaggregation were seen in strains (S-6 and S-8) for *p. aeruginosa*. Strain S-8 showed highest (51%), S-6 showed (48%) and S-7 showed (38%) against *p.aeruginosa* (Figure 3).

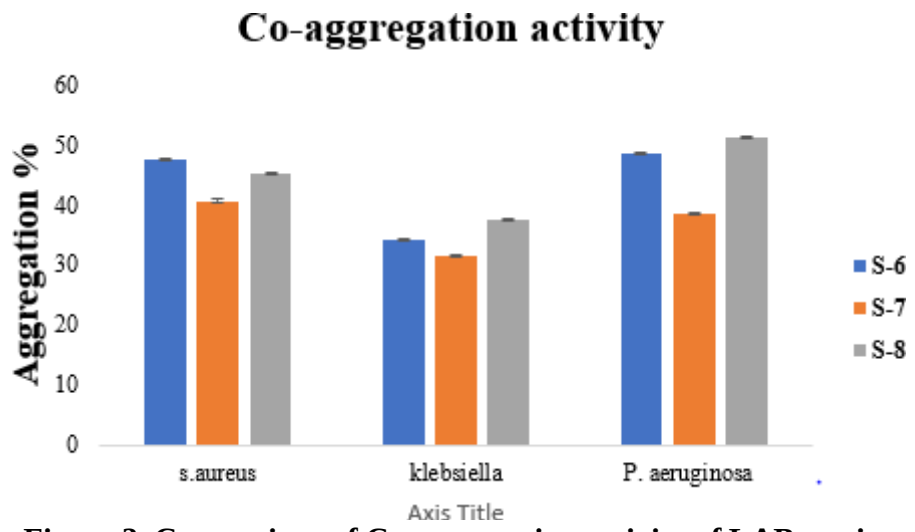

Figure 3. Comparison of Co-aggregation activity of LAB strains

One-way ANOVA (p<0.05) was used to evaluate the data, followed by the Tukeypost hoc test. The results are shown as Mean ± SEM.

Cell surface hydrophobicity

The study used a bacterial adhesion to hydrocarbon assay to evaluate the cell adherence of selected ant ibiotic-sensitive strains, primarily focusing on their hydrophobic properties. In general, tested isolates exhibited high hydrophobicity (>80%) (Figure 4). Notably, isolates S-6 and S-8 displayed particularly high percentages of cell surface hydrophobicity in the results.

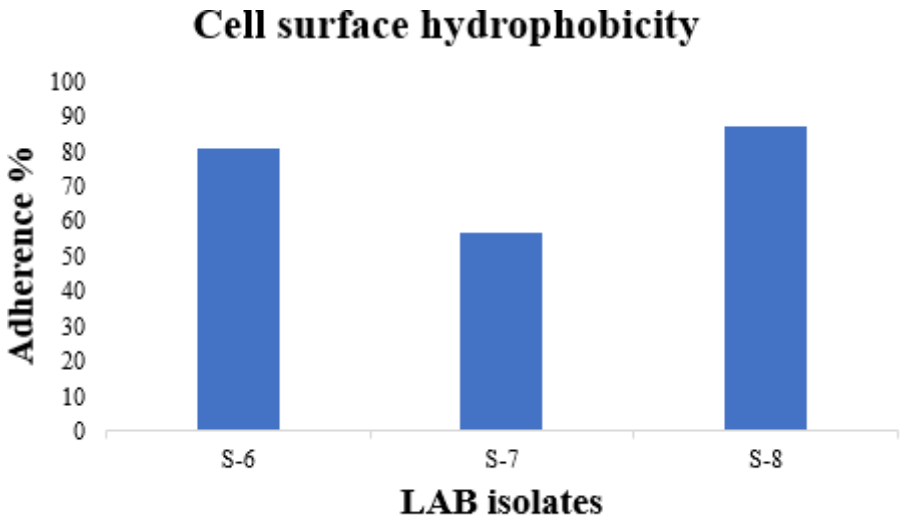

Figure 4. Hydrophobic activity of LAB strains

Antibiofilm activity

In general, *E. coli* and *P. aeruginosa* showed a notable ability to form biofilms.Out of 3 strains isolate S-6 showed 88% antibiofilm activity by *E. coli*, while S-8 resulted in a 70% inhibition (Figure 5). For *P. aeruginosa*, S-6 contributed to a 67%reduction, and S-8 was responsible for a 63% reduction in biofilm formation (Figure 6). These findings highlight the promising potential of LAB strains as probiotics andinhibiting biofilms formed by multidrug-resistant *E. coli* and *P. aeruginosa*.

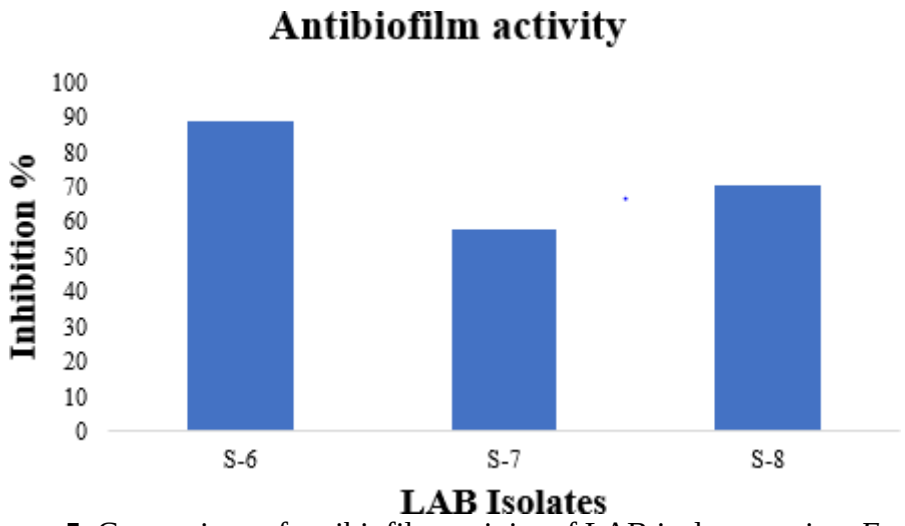

Figure 5. Comparison of antibiofilm activity of LAB isolates against *E. coli*

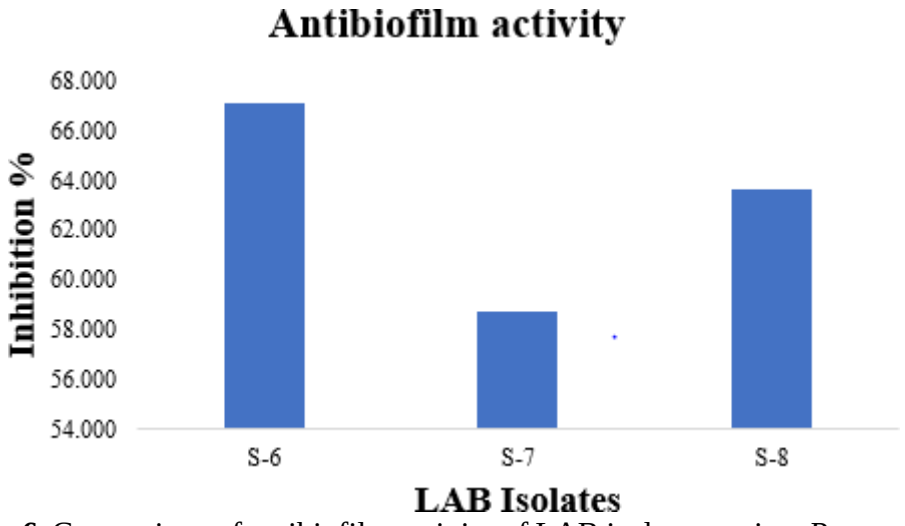

Figure 6. Comparison of antibiofilm activity of LAB isolates against *P. aeruginosa*

In this molecular study, LAB strains S6 and S8 were identified using DNA extraction, PCR amplification of the 16S rRNA gene, and gel electrophoresis. The PCR reaction included components such as 2X Master Mix, forward and reverse primers, and template DNA, followed by agarose gel electrophoresis to verify amplification. The 16S RNA genes were purified with the WizPrep™ Gel/PCR Purification Mini Kit (Cat# W70150-300) and sequenced. Sequencing data were analyzed with BioEdit software, and species identification was performed using NCBI BLAST, revealing S6 as Lacticaseibacillus rhamnosus (accession number: 5974) and S8 as Lactobacillus plantarum (accession number: CE65.8). This comprehensive methodology ensured accurate bacterial species determination.

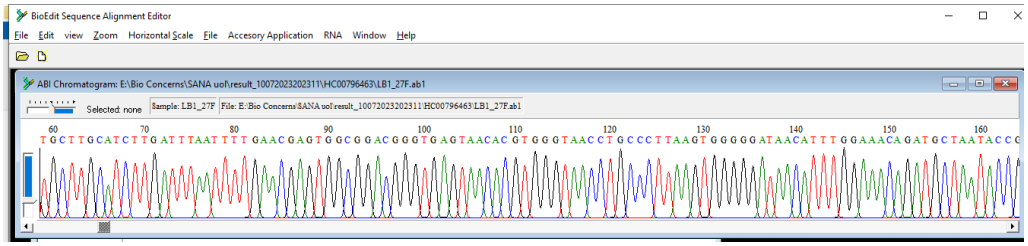

Figure . Analyzation using Bio Edit software

Discussion

In this section of the investigation, two LAB species, *Lactobacillus plantarum* CE56.8 (S-8) and *Lacticaseibacillus rhamnosus* 5974 (S-6), were selected due to theirstrong antibiotic activity against pathogens *(E. coli, S. aureus, Klebsiella,* and *P. aeruginosa*). The probiotic abilities of the isolates were assessed through (a) their ability to withstand varying NaCl, pH, temperature, and bile salt conditions, (b) their adhesion characteristics encompassing biofilm formation, hydrophobicity, and auto- aggregation, (c) their capacity to compete with pathogens via co-aggregation, and (d) asafety evaluation involving their antibiotic susceptibility profile and antimicrobial activity.

One of the desired characteristics of probiotic strains is auto-aggregation, as it helps to inhibit pathogen colonization and lowers the risk of infection (Krausova et al.,2019). After 24 hours of incubation, *Lactobacillus plantarum* CE56.8 (S-8) and *Lacticaseibacillus rhamnosus* 5974 (S-6) displayed the highest levels of auto- aggregation, with >70%.

Based on the results of auto-aggregation assessment, it is possible that *Lactobacillus plantarum* strain CE56.8 (S-8) and *Lacticaseibacillus rhamnosus* strain 5974 (S-6) could form a strong barrier by continuously producing gut biofilm.According to Sannathimmappa et al. in 2021, such a biofilm has the potential to act asan inhibitor against pathogen colonization. These findings are consistent with the findings of Dlamini et al. in 2019, who discovered that Lactobacillus isolates have a significant auto-aggregation capacity of 70%.

As defined by Palencia et al. in 2022, Co-aggregation is a form of interaction with pathogens that functions to eliminate these pathogens within the gastrointestinal tract (GIT) and disrupt their capacity to adhere to host tissues. *Lactobacillus plantarum* CE56.8 (S-8) showed highest (51%) and *Lacticaseibacillus rhamnosus* 5974 (S-6) showed (48%) against *p. aeruginosa*. In a recent investigation, According to Shazadi etal. (2002), *L. reuteri*-MT180537 performed best (58%) against *E. faecalis*-MW051601.

Probiotics play a key role in the development of a beneficial biofilm by populating the epithelium, to build a physical barrier to ward off pathogens. Additionally, according to Campana et al. (2017), the ability of LAB strains to maintaintheir antimicrobial properties over time would seem to be consistent with their ability to adhere to the epithelium. The strains studied were able to producing biofilm, but *Lactobacillus plantarum* strain CE56.8 (S-8) and *Lacticaseibacillus rhamnosus* strain 5974 (S-6) excelled at producing biofilm. This ability allowed them to withstand unfavorable environmental conditions, resulting in successful colonization and population maintenance (Salas-Jara et al., 2016). In a prior investigation, lactobacillusfermentum shown potent biofilm production against *p. aeruginosa* (Shokri et al., 2018)

Probiotics with hydrophobic qualities can greatly improve their host-microbe interaction since mucus, the host's first line of defense, is hydrophobic naturally. Specific cell wall components are supported in their second stage adherence by this primary interaction (Arena et al., 2017). If the H-index is at least 30%–40%, microorganisms can be categorized as hydrophobic; however, microorganisms with an Hindex of 10%–15% are not hydrophobic and are unable to adhere to gut mucosa (Shazadi and Arshad, 2022). According to our findings, the two strains with the highest hydrophobicity (>80%) were *Lactobacillus plantarum* strain CE56.8 (S-8) and *Lacticaseibacillus rhamnosus* strain 5974 (S-6). The hydrophobicity tests conducted in this study demonstrate that these strains have strong gut mucosal interactions, which may have an effect on auto-aggregation. The *L. rhamnosus* strain isolated from breast milk exhibits a 69% hydrophobicity to xylene, following previous investigation (Rajoka et al., 2017).

When determining a strain as a potential probiotic, safety assessment in terms of antimicrobial and antibiotic resistance is one of the most significant considerations that must be primarily addressed (Roe et al., 2022). International standards and guidelines (FEEDAP) were used to determine the antibiotic resistance profile (Additives and Feed, 2012). Results showed that all strains were sensitive

to erythromycin and tetracycline and resistant to streptomycin and vancomycin. Lactobacilli isolated from various sources have been found to be resistant to vancomycin and chloramphenicol in a number of investigations (Akpnar Kankaya andTuncer, 2020).

According to de Melo Pereira et al. (2018), antibacterial activity was the primary goal and an important selection criterion for the identification of innovative and effective probiotics. Pathogenic strains of *E. coli, S. aureus, P. aeruginosa,* and *Klebsiella* showed antagonistic action against isolated LAB strains from cockroach gut.07 out of the 8 strains that were identified showed strong to moderate inhibitory zonesagainst *E. coli*. These findings could be attributed to probiotic bacteria, which line the colon and naturally protect it against pathogenic strains. Probiotic bacteria are beneficial to human health. According to earlier research (Ugi Petrovi et al., 2020), LABs displayed varying ZI against *E. coli* that ranged from 12 to 30 mm.

Overall, this research has explored promising possibilities in the domain of beneficial bacteria, including lactic acid bacteria isolated from the gut of cockroach andlays the foundation for future investigations

Conclusion

The investigated LAB strains such as *Lactobacillus plantarum* CE56.8 and *Lacticaseibacillus rhamnosus* 5974 in this study satisfied several requirements for potential use as probiotic microbes. These included the capacity to adhere to hydrocarbons, the ability to auto- and co-aggregate, and a susceptibility to certain antibiotics. Moreover, these strains showed the ability to prevent the growth of biofilms. These results offer hope in addressing antibiotic resistance and improving healthcare outcomes. To fully realize the potential of these probiotics, it is important to conduct clinical trials, assess safety, optimize formulations, and establish regulatory guidelines. These steps will pave the way for the development of effective LAB-based therapeutics for biofilm-related infections, ultimately reducing healthcarecosts and enhancing patient care.

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