

**Antibiofilm Activity of Probiotic Strains against  
*Aggregatibacter actinomycetemcomitans*.**

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**Abstract**

*Aggregatibacter actinomycetemcomitans* is a significant agent for periodontal disease. It develops biofilms on gingival plaque and promotes chronic pro-inflammatory response to periodontal tissues. As bacterial cells within the biofilm, this pathogen successfully eluded the human immune system by preventing the uptake of biofilm cells by phagocytic cells. Antibiotic-resistant biofilms might harm the environment, increase mortality, and add to the cost of healthcare. Anti-biofilms are necessary to prevent the growth of biofilms. It has been demonstrated that probiotics change how immune system cells produce cytokines. Therefore, the efficacy of five probiotic lactobacillus strains to prevent *A. actinomycetemcomitans* from growing bacteria and forming biofilms was evaluated. The organisms were incubated in anaerobic conditions at 37°C for a certain period. The antimicrobial test was done using the lawn method and measuring the size of the inhibition zone produced by the probiotic spots for each probiotic strain. Tetracycline antibiotic disc was used as a positive control to compare the size of the inhibition zone. The antibiofilm activity was performed using probiotic strains at their exponential and stationary phases against the exponential phase of *A. actinomycetemcomitans*. Our finding showed the probiotic lactobacillus could inhibit the growth of *A. actinomycetemcomitans*. The highest inhibition was demonstrated by *L. johnsonii* NBRC 13952. Besides, all probiotic lactobacillus used for this study significantly ( $p < 0.05$ ) inhibited the *A. actinomycetemcomitans* biofilm using both stationary and exponential phases. In conclusion, probiotic lactobacillus might be a potential agent that can be used to counteract the periodontal pathogen biofilm. Further study should be done to elucidate the antibiofilm mechanism, and several trials of the probiotic lactobacilli against multiple species of biofilms might be helpful in evaluating their effectiveness.

**Keywords**

Probiotics, periodontal pathogen, anti-bacterial, anti-biofilm, stationary phase, exponential phase.

**Introduction**

*Aggregatibacter actinomycetemcomitans*, or *Actinobacillus actinomycetemcomitans*, exhibits a notable prevalence in individuals with aggressive forms of periodontitis. It is a Gram-negative and non-motile bacteria that is closely related to localized aggressive periodontitis (LAP) and chronic inflammatory response to the periodontal area, leading to tooth loss in adolescents. Its colonization area is commonly at the subgingival plaque around the gum line, precisely in the periodontal pockets. These pockets are spaces between the tooth and the surrounding gum tissues. The space is shallow in periodontal health, but in conditions like periodontitis, it deepens due to inflammation and tissue destruction. *A.*

*actinomycetemcomitans* thrive in anaerobic or microaerophilic conditions, making the subgingival environment ideal for colonization. Biofilms are attached and embedded in a self-generated extracellular matrix of mono and polysaccharides, glycoproteins, glycolipids, extracellular DNA, and minerals. It is defined as a well-organized microbial community with water acting like a glue that facilitates cell and cell-matrix interactions [1]. *A. actinomycetemcomitans* generate diverse virulence factors. Many of the serotype B strains of *A. actinomycetemcomitans* were isolated from Localized Juvenile Periodontitis (LJP). It is characterized by rapid bone loss, affecting young adults aged 25 to 35 [2]. The biofilms that form resist antibiotic treatment and the host's defenses. Paradoxically, the high doses of antibiotics used clinically to treat biofilms also contribute to developing antibiotic-resistant strains. Furthermore, the research found that some bacteria within biofilms, so-called 'persistent cells', are dormant mutants exhibiting antibiotic resistance that can become active when treatment is discontinued [3]. Furthermore, the periodontal pathogen can express several virulence factors that enhance the pro-inflammatory responses, such as leukotoxin, cytolethal distending toxin (Cdt), lipopolysaccharide (LPS), bone resorption-inducing toxins, and epitheliotoxin [4].

Using probiotics as antimicrobial and antibiofilm agents presents a promising strategy to address antibiotic resistance. The inherent antagonistic properties exhibited by probiotic strains against pathogenic colonization form the foundational basis for investigating their potential efficacy in combating chronic and biofilm-related infections. Probiotics exhibit significant potential for promoting oral health and combating biofilm formation, which is crucial in preventing oral diseases. Probiotic bacteria, particularly strains of *Lactobacillus* and *Bifidobacterium*, have been shown to exert beneficial effects in the oral cavity by inhibiting the growth of pathogenic bacteria, reducing inflammation, and maintaining a balanced microbial environment [5]. Moreover, probiotics can interfere with the formation of dental biofilms, which are communities of bacteria encased in a protective matrix of extracellular polymeric substances. This biofilm interference can be attributed to the ability of probiotics to produce antimicrobial substances, compete for adhesion sites, and modulate the local immune response [6]. Research indicates that incorporating probiotics into oral care products or as dietary supplements may offer a natural and preventive approach to oral health by promoting a healthier oral microbiota and disrupting the formation of biofilms associated with dental plaque and caries [7]. As the understanding of the intricate interactions within the oral microbiome expands, harnessing the potential of probiotics stands out as a promising strategy to support oral health and combat the challenges posed by biofilm-associated oral diseases.

In a prior investigation [8], our research demonstrated the degradation capabilities of the probiotic *Lactobacillus* against matured biofilms of clinical strains of *A. actinomycetemcomitans*. Furthermore, our earlier study revealed probiotic strains' anti-biofilm activity by impeding clinical strains' biofilm formation [9].

The present study focuses on the ATCC strain of *A. actinomycetemcomitans*, providing complementary and additional insights into its activity compared to our previous research. The impact of probiotic strains was evaluated concerning both anti-bacterial and anti-biofilm activities, specifically targeting the stationary and exponential growth phases of the probiotic strains against the exponential phase of *A. actinomycetemcomitans* ATCC 29522.

## Methods

### Preparation of Samples

The periodontal pathogen species, *A. actinomycetemcomitans* ATCC 29522 was grown in Brain Heart Infusion Agar (BHI). Whereas five probiotics lactobacilli consisted of *L. casei* subspecies *rhamnosus* NBRC 3831, *L. fermentum* JCM 1137, *L. fermentum* NBRC 15885, *L. casei* NBRC 15883, and *L. johnsonii* NBRC 13952 were grown on and De Man, Rogosa, and Sharpe agar (MRS) from the glycerol storage to form colonies. The colonies of the organism were then inoculated into Brain Heart Infusion (BHI) and De Man, Rogosa, and Sharpe (MRS) broth prior proceed to antibacterial and anti-biofilm assay.

### **Agar Spot-on-lawn Assay**

The antibacterial activity of the probiotic lactobacilli against *A. actinomycetemcomitans* was evaluated using spot-on-lawn method. The probiotic lactobacilli strains were spotted on MRS agar. After that, the agar was incubated for 24 hours at 37°C in anaerobic conditions. Later, 200µL of the *A. actinomycetemcomitans* inoculum was mixed into BHI soft agar (0.7%) and poured onto the MRS agar plate with the probiotic spots. After that, the plates were incubated anaerobically for 24 hours at 37°C, and the inhibition zones were measured. A positive result was indicated by a clear zone of more than 1 mm around the spot [10], and according to Shokryazdan et al. the zones of inhibition greater than 20 mm, 10–20 mm, and less than 10 mm were considered strong, moderate, and low inhibition, respectively.

### **Biofilm Inhibition Assay**

The probiotic lactobacilli and a strain of *A. actinomycetemcomitans* were cultured hours prior to conducting the biofilm inhibition assay, based on preliminary growth phase data.[9] A 1:1 ratio of *A. actinomycetemcomitans*'s suspension to probiotic lactobacilli's was utilized for the co-culture assay. In a 96-well plate, 100 µL of each probiotic *Lactobacillus* suspension was co-cultured with 100 µL of *A. actinomycetemcomitans* suspension. The positive control involved adding 100 µL of *A. actinomycetemcomitans* suspension to 100 µL of BHI broth, while the negative control used BHI broth without bacteria. Under anaerobic conditions, the 96-well plates were incubated at 37 °C for 24, 48, and 72 hours to observe *A. actinomycetemcomitans* biofilm formation in the presence of probiotic strains of *Lactobacillus*.

The pH of the biofilm inhibition assay was measured to assess the impact of an acidic environment on biofilm inhibition activity. The pH values of wells with and without probiotic lactobacillus in both exponential and stationary phases, co-cultured with *A. actinomycetemcomitans*, were measured using a LAQUAtwin compact pH meter (Horiba Ltd, Kyoto, Japan).

### **Biofilm Quantification**

By modifying the method by Saulnier et al. [11], the biofilm microtiter plate was washed three times by immersing it in distilled water. This washing removes loosely attached cells or media that the crystal violet may stain. To determine the total biofilm mass, 200 µL of 0.1% crystal violet (w/v) was added to each well and allowed to dissolve for 30 minutes. Then, the plates were gently rinsed using distilled water and air-dried in a 37°C incubator for 15 minutes. Residual biofilms was visualized via photographs. Stained biofilms were then dissolved with 200 µL of 95% ethanol to each well for 30 minutes. Plates were read using an ELISA microplate reader at an absorbance of 492 nm wavelength using a Tecan microplate reader. The software used to measure absorbance is Magellan.

### **Percentage of Biofilm Inhibition calculation**

To determine the exact value of the biofilm-inhibition of the probiotic lactobacillus against *A. actinomycetemcomitans*, the percentage of the anti-biofilm activity was calculated using the following formula [10]:

$$\% \text{ Inhibition} = \left[ \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right] \times 100$$

### **Statistical Analysis**

Quantitative analysis of biofilm formation and statistical significance were determined using IBM SPSS statistics. The mean difference between groups was estimated using a paired *t*-test. A *p*-value<0.05 was considered statistically significant. Data collected was represented as mean values ± standard deviation.

## Result

### Inhibition Zone

The agar spot assay assessed the antibacterial activity of probiotic lactobacilli against the *A. actinomycetemcomitans* by measuring the inhibition zones. The result of the inhibition zone produced by the five probiotic lactobacillus strains against *A. actinomycetemcomitans* ATCC 29522 is shown in Table 1. The activity was compared with the positive control, tetracycline antibiotic disc.

**Table 1: The size of the inhibition zone produced by probiotic lactobacillus and antibiotic disk control.**

Probiotic Lactobacillus	Inhibition Zone (cm ± SD)	
	Control	Spot
<i>L. fermentum</i> NBRC 15885	2.1 ± 0.141	0.90 ± 0.096
<i>L. fermentum</i> JCM 1137	2.3 ± 0.115	0.75 ± 0.100
<i>L. casei</i> NBRC 15883	2.0 ± 0.141	0.88 ± 0.050
<i>L. casei subspecies rhamnosus</i> NBRC 3831	2.1 ± 0.126	0.75 ± 0.058
<i>L. johnsonii</i> NBRC 13952	2.1 ± 0.096	0.65 ± 0.058

Based on the result shown in Table 1, *L. fermentum* NBRC 15885 (LF85) was the most significant in inhibiting the growth of the *A. actinomycetemcomitans*. The activity was followed by *L. casei* NBRC 15883 (LC83), *L. fermentum* JCM 1137 (LF37), and *L. casei subspecies rhamnosus* NBRC 3831 (LCR31). *L. johnsonii* NBRC 13952 (LJ52) was the least significant in inhibiting the growth of *A. actinomycetemcomitans*. The probiotic spots were in the low inhibition range in inhibiting the growth of *A. actinomycetemcomitans* compared to the inhibition zone produced by the tetracycline antibiotic disc control, which can be categorized in potent inhibition in which the size ranging from 2.0 cm to 2.3 cm.

### Influence of exponential and stationary phases of probiotic lactobacilli on the anti-biofilm activity.

The anti-biofilm activity of probiotic lactobacilli against *A. actinomycetemcomitans* was compared between probiotic lactobacilli's exponential and stationary phases.

**Table 2: The percentage of biofilm inhibition of the probiotic's exponential and stationary phase against *A. actinomycetemcomitans***

Incubation Time	Probiotic Strains	Biofilm Inhibition (%)	
		Exponential	Stationary
24 Hours	LC83	68.18	68.18
	LF85	68.18	68.18
	LF37	63.64	63.64
	LCR31	72.73	72.73
	LJ52	68.18	68.18
48 Hours	LC83	71.43	46.43
	LF85	71.43	64.29
	LF37	67.86	64.29
	LCR31	57.14	71.43
	LJ52	71.43	67.86
72 Hours	LC83	69.57	47.83
	LF85	69.57	60.87
	LF37	69.57	56.52
	LCR31	69.57	65.22
	LJ52	73.91	65.22

After 24 hours of incubation, *L. casei* NBRC 15883 (LC83) in both exponential and stationary growth phases showed a similar percentage of biofilm inhibition (68.18%). However, during 48 hours of incubation, LC83 in the exponential growth phase showed a higher percentage of biofilm inhibition (71.43%) than in the stationary phase (46.43). The result of biofilm inhibition by LC83 in 72 hours of incubation also showed a higher inhibition by the exponential growth phase with 69.57%. Meanwhile, the stationary growth phase was only 47.83%.

Next, *L. fermentum* NBRC 15885 (LF85) in 24 hours of incubation time showed a similar value of percentage in biofilm inhibition for both exponential and stationary growth phases (68.18%) meanwhile, for 48 hours of incubation, LF85 in the exponential growth phase showed the value of 71.43% and stationary growth phase showed 64.29% of biofilm inhibition. LF85 in the exponential growth phase showed 69.57% biofilm inhibition, slightly higher than stationary LF85 with 60.87% in 72 hours of incubation.

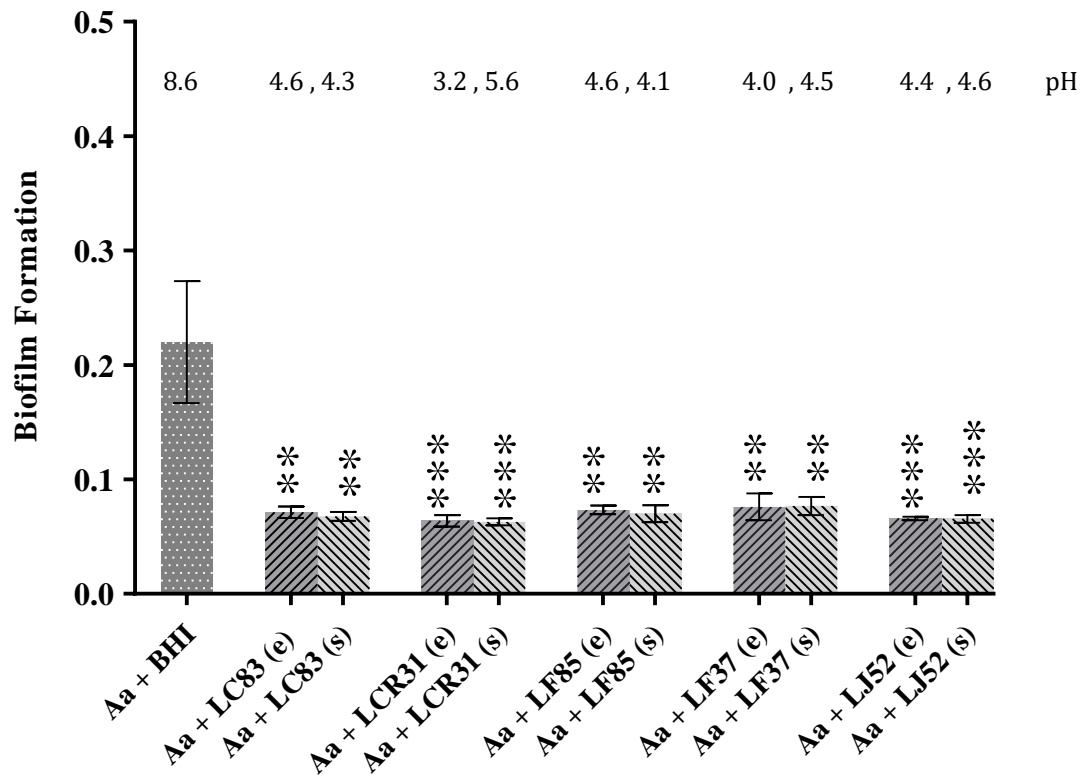
*L. fermentum* JCM 1137 (LF37) showed the exact value of biofilm inhibition (63.64%) in both exponential and stationary growth phases during the 24 hours of incubation. After 48 hours of incubation, the biofilm inhibition for the strain was slightly increased, with 67.86% for the exponential growth phases of the probiotic and 64.29% by stationary growth of the probiotic. The biofilm inhibition percentage also increased to 69.57% for the exponential growth of probiotic strain LF37 after 72 hours of incubation. However, the inhibition reduced to 56.52% for the stationary growth phase.

Other than that, for 24 hours of incubation, *L. casei* subspecies *rhannosus* NBRC 3831 (LCR31) also showed a similar percentage value of biofilm inhibition for both exponential and stationary growth phases with 72.73%. During 48 hours of incubation, the exponential growth of LCR31 decreased in the biofilm inhibition to 57.14. Meanwhile, the stationary phase only slightly reduced inhibition to 71.43%. After 72 hours of incubation, LCR31 in the exponential phase showed a higher value than 48 hours with the value of 69.57% meanwhile, biofilm inhibition by stationary LCR31 during 72 hours of incubation is lower compared to 48 hours of incubation.

Lastly, for *L. johnsonii* NBRC 13952 (LJ52), after 24 hours of incubation, the percentage of biofilm inhibition by exponential and stationary growth phases showed a similar value, 68.18% meanwhile, in 48 hours of incubation, the exponential LJ52 inhibited the biofilm formation by 71.43%, and the stationary showed 67.86% of inhibition. The exponential LJ52 increase in the biofilm inhibition to 73.91% while the stationary decreased to 65.22%.

#### **Biofilm inhibition activity after 24, 48, and 72 hours incubation.**

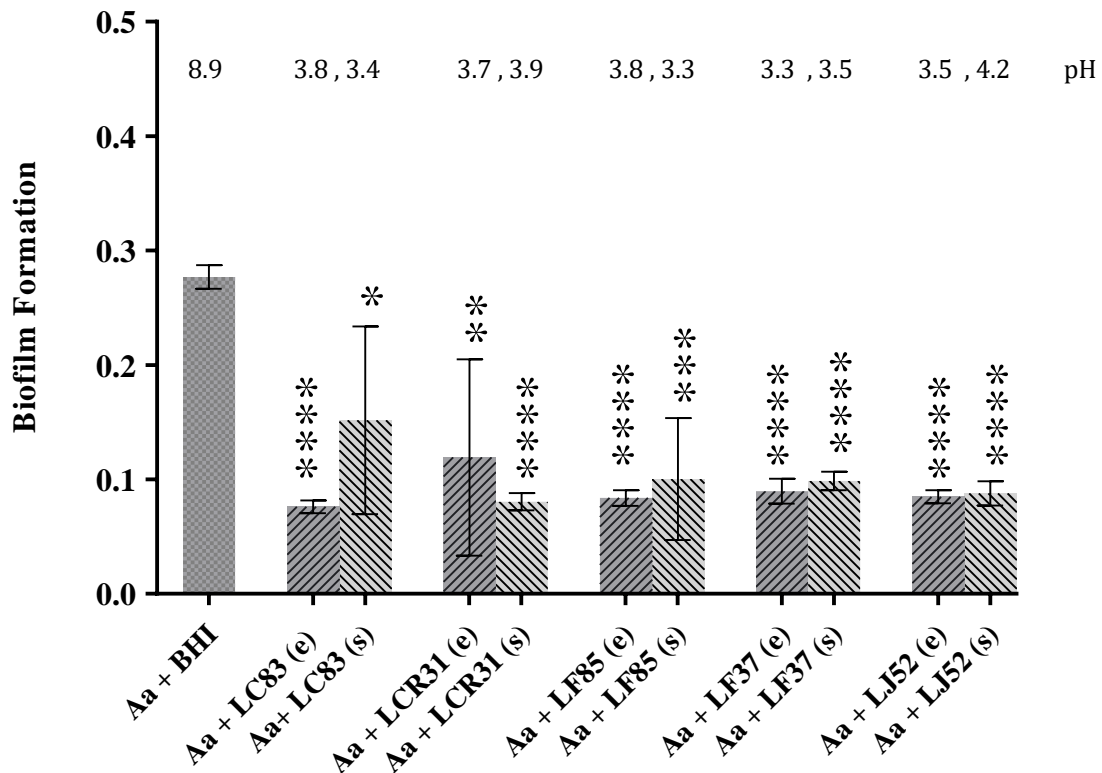
The comparison between the positive control sample containing *A. actinomycetemcomitans*, ATCC29522 strain in an exponential growth phase in BHI broth, and the treatment group using the five probiotic lactobacilli in exponential and stationary growth phases with the *A. actinomycetemcomitans*, ATCC29522 strain were shown in Graph 1 (24hours), Graph 2 (48 hours), and Graph 3 (72 hours).



**Figure 1: The biofilm formation of *A. actinomycetemcomitans* in BHI (control) and *A. actinomycetemcomitans* co-cultured with probiotic strain (treatment) for 24 hours of incubation. Bars represent the mean; error bars represent the standard deviation, and significance was measured using paired T-test (\*\*\*\* =  $p < 0.0001$ ), (\*\*\*) =  $p < 0.0021$ ), (\*\*) =  $p < 0.0332$ ), and (\*) =  $p < 0.05$ ).**

The biofilm formed was measured to determine the biofilm inhibition by the probiotic strains. After 24-hours of incubation, *L. casei* subspecies *rhamnosus* NBRC 3831 and *L. johnsonii* NBRC 13952 in both exponential and stationary growth phases showed the most significant antibiofilm activity against *A. actinomycetemcomitans*.

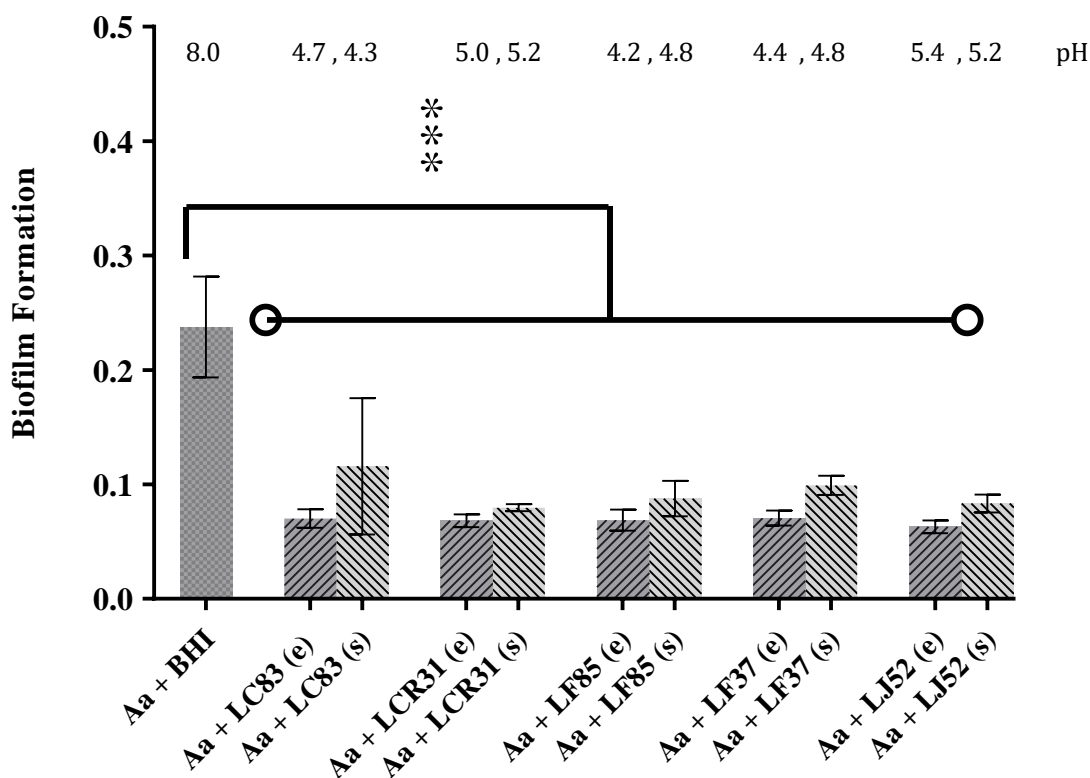
The *L. casei* subspecies *rhamnosus* NBRC 3831 (LCR 31) and *L. johnsonii* NBRC 13952 (LJ 52) showed significantly lower biofilm formation values compared to others with LCR31 (e) ( $0.06 \pm 0.005$ ), LCR31 (s) ( $0.06 \pm 0.003$ ), LJ52 (e) ( $0.07 \pm 0.001$ ) and LJ52 (s) ( $0.07 \pm 0.003$ ) compared with other probiotic strains with the positive control value ( $0.22 \pm 0.053$ ). Statistical analysis of the significant difference between exponential and stationary phases showed no significant difference ( $p\text{-value} > 0.05$ ). Interestingly, the antibiofilm activity indicated by the absorbance value was similar between the exponential and stationary of the probiotic strains.



**Figure 2: The biofilm formation of *A. actinomycetecomitans* (Aa) in BHI (control) and *A. actinomycetecomitans* co-cultured with probiotic strain (treatment) for 48 hours of incubation. Bars represent the mean, error bars represent the standard deviation, and significance was measured using paired T-test (\*\*\*\* =  $p < 0.0001$ ), (\*\*\*) =  $p < 0.0021$ , (\*\*) =  $p < 0.0332$  and (\*) =  $p < 0.05$ ).**

After 48 hours of incubation, the *L. johnsonii* NBRC 13952 (exponential & stationary), *L. fermentum* JCM 1137 (exponential & stationary), *L. fermentum* NBRC 15885 (exponential), *L. casei* subspecies *rhamnosus* NBRC 3831 (stationary) and *L. casei* NBRC 15883 (exponential) demonstrated significant antibiofilm activity with the (mean  $\pm$  SD) value; LJ52(e) ( $0.08 \pm 0.006$ ), LJ52(s) ( $0.09 \pm 0.011$ ), LF37(e) ( $0.09 \pm 0.011$ ), LF37 (s) ( $0.10 \pm 0.008$ ), LF85(e) ( $0.08 \pm 0.007$ ), LCR31(s) ( $0.08 \pm 0.007$ ) and LC83(e) ( $0.08 \pm 0.006$ ) compared with the positive control ( $0.28 \pm 0.010$ ).

After 72 hours of incubation, our finding showed that anti-biofilm activity was significantly higher for all probiotic strains. Consistent with 24 and 48 hours incubation, no significant difference was found in the anti-biofilm activity between the exponential and stationary phases for all probiotic strains used in this study.



**Figure 3: The biofilm formation of *A. actinomycetemcomitans* in BHI alone (control) and *A. actinomycetemcomitans* co-cultured with probiotic strain (treatment) for 72 hours of incubation. Bars represent the mean, error bars represent the standard deviation, and significance was measured using paired T-test (\*\*\*\* =  $p < 0.0001$ ), (\*\*\*) =  $p < 0.0021$ ), (\*\*) =  $p < 0.0332$ ), and (\*) =  $p < 0.05$ ).**

## Discussion

*A. actinomycetemcomitans* serves as the causative pathogen for periodontal disease, wherein it establishes biofilm structures on dental plaque along the gingival margin, thereby inducing periodontitis, a chronic inflammatory condition affecting the periodontal tissues. The resultant progressive inflammatory response adversely impacts the structural integrity of dental tissues. Within biofilms, bacterial cells adeptly evade host immune defences by impeding the phagocytic uptake of biofilm cells. Moreover, conventional antibiotic treatments prove ineffectual in combating biofilm-associated infections, necessitating impractical high dosages to achieve biofilm inhibition. This dilemma inadvertently contributes to antibiotic resistance and treatment ineffectiveness. To surmount these challenges, alternative strategies, such as using probiotic strains to impede biofilm growth, warrant exploration.

Each of the five probiotic Lactobacillus strains demonstrated antagonistic activity against *A. actinomycetemcomitans*. The determination of inhibition activity was categorized as strong for diameters  $>20$  mm, moderate for those ranging from 10 to 20 mm, and low for diameters  $<10$  mm [10]. In alignment with the inhibition activity results presented in Table 1, all five strains of probiotic Lactobacillus exhibited low inhibition activity against *A. actinomycetemcomitans*. The observed low inhibition activity of probiotic lactobacilli against *A. actinomycetemcomitans* may suggest the presence of various virulence factors enabling periodontal pathogens to withstand the challenges posed by the probiotic lactobacilli. Probiotic lactobacilli produce diverse metabolites that can influence inhibition activity, including generating toxic compounds such as bacteriocins, lactic acid, and hydrogen peroxide [12]. Lactic acid bacteria generally

produce organic acids, mainly lactate and acetate, thus creating an acidic environment that inhibits pathogenic bacteria. Besides, they synthesize proteins and peptides that can inhibit certain pathogenic strains. Many studies have reported that lactic acid bacteria produce well-characterized inhibitory peptides such as bacteriocins and bacteriolysins [13]. Other than that, organic acids, mainly lactic and acetic acid, hydrogen peroxide, and bacteriocins are the most common antimicrobials reported to be produced by probiotics lactobacilli [14].

Administration of antibiotics during root planning, scaling, or periodontal surgery reliably eliminates *A. actinomycetemcomitans* from periodontal lesions. Unfortunately, nearly all species are protected by the exopolysaccharides (EPS) at the biofilm stage, making antibiotics less effective. Lactic acid bacteria can influence pathogenic bacteria and fungal growth through coaggregation activity, competition for nutrients, production of antimicrobial compounds, and immune response modulation. Furthermore, few studies have shown that lactobacilli can integrate into target biofilms and temporarily colonize the oral cavity while competing with pathogens for adhesion sites [12].

The data obtained in this study confirmed a significant reduction in biofilm formation and a production of inhibition areas around colonies of *A. actinomycetemcomitans* after treatment with different strains of probiotics. In the well containing the probiotic strains of Lactobacillus, the pH value was acidic, ranging from pH 3 to pH 5, which it may be due to lactic acid and other chemical compounds with acidic properties, leading to an acidic environment to kill the *A. actinomycetemcomitans*. In addition, probiotics prevent pathogenic biofilm formation by lowering environmental pH and biofilm biomass [14]. Changes in environment and pH value can also affect biofilm formation in *A. actinomycetemcomitans* [8].

The growth phase of the probiotic strains might influence the biofilm formation activity. However, our findings showed that the difference was not significant. In the exponential phase, the growth rate increases, cell doubling is relatively constant, and stable metabolic activity exists. Whereas, during the stationary phase, bacterial growth starts to slow down and stops due to depletion of the available nutrients and increased concentration of the byproducts compound. Even with a zero-growth rate, cells are still at the stage of metabolically active and produce secondary metabolites. In the stationary phase, metabolite deregulation increases the production of specific metabolites [15].

Bacteriocin is one of the chemicals secreted by the probiotic strain of lactobacillus. Bacteriocins are antibacterial proteins produced during the exponential growth phase and follow the pattern of primary metabolite synthesis. The more cells grow, the faster they utilize nutrients in the medium [12,13]. Various factors can trigger bacterial entry into the stationary phase. This includes restriction of certain essential nutrients, accumulation of toxic by-products, and presence of toxic substances. It is affected by stress factors such as changes in pH, temperature, osmolarity, etc. Once cells enter this stage, it is said to decrease cell size and increase the DNA or protein ratio during migration to the stationary phase. The cells become spherical and smaller, rigid cell envelopes, the cell wall is strongly cross-linked, and membrane fluid is reduced, and cells activate stringency response mechanisms for surviving the catastrophe [16]. All of these factors that occur during the probiotics' exponential and stationary growth phase may be the reason for the rate of biofilm inhibition activity.

A reduction in biofilm formation might indicate the high inhibitory and co-aggregation activity between the probiotic strains and the *A. actinomycetemcomitans*. During a 24-hour culture period, *L. casei subsp. rhamnosus* NBRC 3831 and *L. johnsonii* NBRC 13952 exhibited significant effects in inhibiting the biofilm formation of *A. actinomycetemcomitans* during both exponential and stationary growth phases. During 48 hours of incubation time, the *L. johnsonii* NBRC 13952 (exponential & stationary) also significantly inhibited biofilm formation due to the low value of absorbance representing the biofilm formation. The least significant probiotic was *L. casei* NBRC 15883 (stationary). In 72 hours of incubation, all probiotic strains showed a significant value in inhibiting the biofilm. The result varies between strains, possibly

influenced by factors such as lactic acid concentration, bacteriocin, and other active metabolites. Laakso et al. 2011, [17] stated that *L. plantarum* showed differences in protein expression profiles in multiple anabolic and stress response pathways throughout different developmental stages. Lactobacillus derivatives, such as the supernatant, can also benefit the body. Multiple results suggest that lactobacillus-derived cell-free supernatant (CFS) acts as a biological liquid detergent by reducing surface pathogen adherence. For instance, the biofilm formation of the multidrug-resistant superbugs, namely *Pseudomonas aeruginosa* and *Staphylococcus aureus*, is mitigated by cell-free supernatant of *L. casei*, *L. fermentum*, *L. gasseri*, *L. plantarum*, and *L. salivarius* [14].

The potential anti-biofilm mechanisms of probiotic Lactobacillus against *A. actinomycetemcomitans* involve diverse strategies. Bacteriocin production is crucial, as these antimicrobial peptides produced by Lactobacillus can selectively target and inhibit the growth of *A. actinomycetemcomitans*, disrupting biofilm formation [18]. Additionally, lactic acid production by Lactobacillus leads to the acidification of the microenvironment, creating unfavorable conditions for *A. actinomycetemcomitans* within biofilms and impeding their development [19]. Furthermore, the generation of hydrogen peroxide by probiotic Lactobacillus contributes to its anti-biofilm activity, as hydrogen peroxide possesses antimicrobial properties that can interfere with the structural integrity of *A. actinomycetemcomitans* biofilms. These combined mechanisms highlight the potential of probiotic Lactobacillus to disrupt biofilm formation by *A. actinomycetemcomitans* through targeted antimicrobial and environmental modulation strategies.

## Conclusion

In conclusion, Lactobacillus probiotic strains exhibit discernible antimicrobial and antibiofilm properties against *A. actinomycetemcomitans*. Although the antimicrobial efficacy of probiotic lactobacilli was comparatively lower than that of tetracycline, their anti-biofilm activity demonstrated pronounced effectiveness. This notable effect holds significant potential for combatting biofilm-related infections. Given the limitation of the study focusing solely on one species of periodontal pathogen, further investigations targeting multispecies biofilms would be valuable to present the actual situation of infection and to assess the broader effectiveness of probiotic strains as anti-biofilm agents. Additionally, considering biofilm formation in natural settings involves multiple bacterial species, exploring biofilm-related genes could enhance our understanding of the underlying mechanisms during these activities.

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## Conflict of Interest Disclosure

There is nothing to declare.

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