

Comparative Analysis of Commercial Yogurt Products in Indonesia for Pathogen Growth Inhibition and Biofilm Disruption and Inhibition

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Abstract

The human gut microbiota plays an important role in gastrointestinal health, but pathogenic infections may disrupt microbial balance and cause dysbiosis. Probiotics in fermented dairy products such as yogurt are known to exhibit antimicrobial and antibiofilm properties. This study aimed to evaluate the antibacterial, biofilm disruption, and biofilm inhibition activities of commercial yogurt products available in Indonesia against *E. coli* and *Salmonella* spp. This *in vitro* study used agar well diffusion for antibacterial testing, while biofilm disruption and inhibition assays were conducted using 96-well microplates, crystal violet staining, and microplate reader analysis. Three commercial yogurts (X, Y, and Z) were tested alongside chloramphenicol and sterile aquadest as controls. Data were analyzed using one-way ANOVA and Tukey HSD tests. All yogurt samples exhibited antibacterial activity. Yogurt Y showed the highest inhibition against *E. coli* (7.1 ± 0.2 mm) and *Salmonella* spp. (7.43 ± 0.25 mm). In biofilm disruption assays, yogurt treatments increased biofilm biomass, whereas chloramphenicol reduced preformed biofilms. Conversely, yogurt treatments inhibited biofilm formation, with Yogurt Y showing the strongest inhibition against *E. coli* and Yogurt X against *Salmonella* spp. These findings suggest that commercial yogurts may be more effective in preventing biofilm formation than eliminating mature biofilms.

Keywords: biofilm; lactic acid bacteria; pathogen growth inhibition; probiotics; yogurt

INTRODUCTION

The human gut microbiota refers to the diverse community of microorganisms including bacteria, archaea, fungi, and viruses that inhabit the gastrointestinal tract, particularly the intestines. These microorganisms live in a symbiotic relationship with the human host and play essential roles in digestion, metabolism, immune system development, protection against pathogens, and the maintenance of overall gut and host health (Thursby & Juge, 2017). Problems in gut microbiota health can arise due to the presence or overgrowth of pathogenic bacteria. These pathogens can disrupt the normal balance of beneficial microorganisms (a condition known as dysbiosis), which may lead to inflammation, impaired digestion, weakened immune responses, and various gastrointestinal or systemic diseases (Petersen & Round, 2014).

Two bacteria that are most known to cause problems within the gut are *Salmonella* sp. and *Escherichia coli*. *Escherichia coli* is a gram-negative bacillus responsible for various diarrheal diseases, such as traveler's diarrhea and dysentery. It is the leading cause of uncomplicated cystitis and can also contribute to extraintestinal

infections, including pneumonia, bacteremia, and abdominal conditions like spontaneous bacterial peritonitis. *E. coli* infections impose a considerable burden on both patients and healthcare systems, making early detection and effective treatment essential (Pokharel et al., 2023). *Salmonella* is a foodborne pathogen from the Enterobacteriaceae family that causes gastroenteritis in humans. It can reside in various hosts, including animals, amphibians, and reptiles. Transmission to a healthy individual typically occurs through the ingestion of contaminated food or water (Lamichhane et al., 2024).

To inhibit these pathogens, antibiotics are usually used which are antimicrobial agents that are capable of killing or suppressing the growth of disease-causing microorganisms at relatively low concentrations by targeting specific and essential cellular processes, a property known as selective toxicity. These compounds act by interfering with key physiological functions of microbial cells, such as cell wall synthesis, protein production, or nucleic acid replication, while minimizing harm to the host. Antibiotics are widely used in both human and veterinary medicine as therapeutic tools to treat bacterial infections, supporting the host's immune

system in controlling and eradicating pathogenic microorganisms (Cerf et al. 2009).

However, the use of antibiotics could lead to an imbalance within the gut microbiota as the antibiotics often disrupt the natural balance and diversity of microbial communities in the gastrointestinal tract. While antibiotics are designed to eliminate pathogenic bacteria, they often also affect beneficial commensal microorganisms, leading to a reduction in microbial diversity and abundance. This disruption, known as dysbiosis, can impair essential gut functions such as digestion, vitamin synthesis, and immune regulation (Patangia et al. 2022).

In order to maintain the health of the gut microbiota while fighting off pathogens, probiotics and prebiotics are usually consumed. Probiotics, such as lactic acid bacteria (LAB), are non-pathogenic microorganisms that provide health benefits to the host when consumed in sufficient amounts. At present, extensive research is focused on understanding the molecular mechanisms and practical applications of probiotics. Their proposed modes of action include competing with pathogens for attachment sites, enhancing the intestinal mucosal barrier, modulating the immune system in the gut, and contributing to neurotransmitter production (Latif et al. 2023).

This research aims to evaluate the efficacy in which the commercial yogurt products sold in Indonesia are able to disrupt pathogenic bacteria growth as well as inhibit biofilm formation and disrupt pre-formed biofilm.

MATERIALS AND METHODS

Time and Place of Research

This research was conducted for 8 months (10 September 2025 – 30 April 2026). At the laboratory of Microbiology, Faculty of Biology Universitas Gadjah Mada and Fasilitas Laboratorium Bersama (FALITMA), Faculty of Biology Universitas Gadjah Mada

Yogurt Collection

To obtain the yogurt samples, three yogurts which are the most purchased within the consumers are bought in local supermarkets across Yogyakarta namely yogurt X, Y, and Z all which are strawberry-flavored yogurt.

Bacterial Strain Preparation

To conduct the experiment, bacterial strains are first prepared to be used in the experiments. The pathogen bacteria is prepared by sub-culturing the bacteria. The bacteria is first inoculated from an existing culture to a nutrient broth which is then incubated at 37°C overnight. The new culture is then spread on an agar plate using a drygalski then is incubated again at 37°C overnight. Afterwards the single colony formed from the spread plate is inoculated to a tube of slant agar as a storage culture for future experiments.

Pathogen Inhibition Assay

Initially, the pathogen bacterial cultures are prepared in individual NB media at 37°C overnight. The bacterial concentration is then standardized to 10⁷ CFU/mL using Optical density (OD) at 600nm wavelength measurements. Next, the experiments are set up by streaking the pathogens in an agar plate using a drygalski, then 3 wells are made in the agar plate using a cork borer. The wells are then filled with the yogurt as treatment, C+ chloramphenicol as a positive control, and aquadest as a negative control. Then the clear zone is then measured using a caliper

Biofilm Disruption Assay

Biofilm disruption by *Escherichia coli* and *Salmonella* spp. was evaluated using a crystal violet staining assay in 96-well microplates. Both bacteria were first inoculated into fresh tryptic soy broth (TSB) and incubated for 18 hours. The resulting cultures were adjusted to an OD of 0.1 using a spectrophotometer. After that, 100 µL of the standardized bacterial culture+100 µL of fresh TSB was added to the designated wells. The plate was then incubated at 37°C for 24 hours to allow biofilm formation. Empty wells were filled with sterile aquadest to minimize edge effects. After the incubation, the culture medium was carefully aspirated, and each well was gently washed three times with phosphate-buffered saline (PBS) to remove non-adherent cells. Biofilm disruption was then performed by adding 200 µL of yogurt supernatant as the treatment. Negative control wells received 200 µL of fresh TSB only, while positive control wells received 200 µL of a chloramphenicol solution. The plates were subsequently incubated again at 37°C for 24 hours. After the second incubation, the treatment solutions were removed, and the wells were washed two to three times with PBS. The remaining attached biofilm was fixed by adding 200 µL of 96% ethanol per well and incubating for 10–15 minutes, after which the ethanol was discarded and the plate was air-dried. Subsequently, 200 µL of 0.1% crystal violet solution was added to each well and incubated at room temperature for 10–15 minutes. Excess stain was removed, and the wells were rinsed gently with sterile distilled water three to five times until the wash solution became clear. The plate was then air-dried, and the bound crystal violet was solubilized by adding 200 µL of 96% ethanol to each well and incubating for 10–15 minutes. Biofilm biomass was quantified by measuring the absorbance at 570 nm using a microplate reader.

Biofilm Inhibition Assay

Biofilm formation by *Escherichia coli* and *Salmonella* spp. was done by inoculating both bacteria into a tube of fresh TSB which is then incubated for 18 hours. The incubated media is then adjusted to reach an OD of 0.1 by using a spectrophotometer. The calibrated culture is

then inoculated into the designated wells in the 96-well microplate with the formula of 100 μ L culture+100 μ L fresh TSB for the negative control, followed by 100 μ L culture+100 μ L yogurt supernatant as the treatment, and finally 100 μ L culture+100 μ L C+ chloramphenicol mixture for the positive control while the empty wells are filled with sterile aquadest in order to reduce the edge effects in the microplate. The inoculated well plates are then incubated at 37°C for 24 hours to form biofilm. After the incubation, the culture medium was carefully aspirated, and each well was gently washed two to three times with phosphate-buffered saline (PBS) to remove non-adherent cells. The remaining attached biofilm was fixed by adding 200 μ L of 96% ethanol per well and incubating for 10–15 minutes, after which the ethanol was discarded and the plate was air-dried. After the plates have dried, 200 μ L of 0.1% crystal violet solution was added to each well and incubated at room temperature for 10–15 minutes. Excess stain was removed, and the wells were rinsed gently with sterile distilled water three to five times until the wash solution became clear. The plate was then air-dried, and the bound crystal violet was solubilized by adding 200 μ L of 96% ethanol to each well and incubating for 10–15 minutes. Biofilm biomass was quantified by measuring the absorbance at 570 nm using a microplate reader.

Data Analysis

The data analysis for the antimicrobial activity assay will be performed by calculating the mean and standard deviation of inhibition zone diameters, followed by one-way ANOVA to compare differences among yogurt treatment, positive control (chloramphenicol), and negative control (aquadest), with post hoc Tukey's test applied when significant differences are detected; all analyses will be conducted using SPSS. Biofilm inhibition and disruption data obtained from OD570 measurements will be expressed as mean $\pm$ standard deviation and analyzed using a one-way ANOVA which was performed using SPSS.

RESULTS AND DISCUSSION

Pathogen Growth Inhibition

The measurement of pathogen growth inhibition of various yogurt was conducted using the agar well diffusion method, in which the clear zones formed around each well was measured. A few samples were tested using this method which includes Yogurt X, Yogurt Y, and Yogurt Z, as well as chloramphenicol as the positive control and sterile aquadest as the negative control, to assess the effectivity of each yogurt and compare them with each other as well as the negative and positive control. The results of the analysis are as shown in Table 1:

Table 1. Results of Pathogen Growth Inhibition Test for *E. Coli* and *Salmonella* spp.

Strain	Inhibition Zone (mm)				
	Yogurt			Control	
	X	Y	Z	+	-
<i>E. coli</i>	6.9 $\pm$ 0.1 ^b	7.1 $\pm$ 0.2 ^b	6.4 $\pm$ 0.1 ^b	28.37 $\pm$ 1.92 ^c	0 $\pm$ 0.1 ^a
<i>Salmonella</i> spp.	6.9 $\pm$ 0.27 ^b	7.43 $\pm$ 0.25 ^b	7.1 $\pm$ 0.1 ^b	28.16 $\pm$ 1.65 ^c	0 $\pm$ 0.1 ^a

The results show that when it comes to the inhibition of *E. coli* yogurt Y has the highest inhibition zone with a mean of 7.1 $\pm$ 0.2 mm followed by yogurt X with a mean of 6.9 $\pm$ 0.1 mm, and yogurt Z being the lowest with a mean of 6.4 $\pm$ 0.1 mm. All the yogurts are shown to be able to inhibit *E. coli* growth, however the analytical results from SPSS shows that the difference in inhibition is insignificant ($F(4, 10) = 467.595$, $p < .001$). For the *Salmonella* spp. inhibition on the other hand, yogurt Y still proves to be the most effective, showing a mean of 7.43 $\pm$ 0.25 mm of inhibition zone, followed by Yogurt Z which shows an inhibition zone of 7.1 $\pm$ 0.1 mm, and lastly comes Yogurt X with the lowest inhibition zone sitting at 6.9 $\pm$ 0.27 mm. Same with the *E. coli* the yogurts are shown to be able to inhibit *Salmonella* spp. growth, however the analytical results from SPSS shows that the difference in inhibition is insignificant ($F(4, 10) = 594.469$, $p < .001$). The activity of the antibiotics on the

other hand shows a higher level of inhibition with the mean for *E. coli* across all repetitions being 28.37 $\pm$ 1.92 mm and the mean for *Salmonella* spp. being 28.16 $\pm$ 1.65 mm.

From the results it can be seen that there is a clear significant bacteria growth inhibition as a result of the treatment. This result is in accordance with theory, all three yogurts used for the treatment in this experiment includes bacteria *Lactobacillus delbrueckii* subsp. *Bulgaricus* (*L. Bulgaricus*) as one of the main cultures used within the yogurt. This bacteria in particular inhibits pathogenic growth through several complementary mechanisms. One of the primary mechanisms is the production of organic acids, particularly lactic acid (Bai et al. 2024), which lowers the surrounding pH and creates an unfavorable environment for enteric pathogens such as *Escherichia coli* and *Salmonella* spp. In acidic conditions, disruption of the proton motive force causes

ions, including protons, to leak across the membrane, resulting in a decrease in cytoplasmic pH. This disturbance induces various detrimental effects in bacterial cells, and if the physiological pH cannot be reestablished, cellular components may become damaged, including the eventual breakdown of the cytoplasmic membrane (Porto-Figueira et al. 2022).

In addition to organic acid production which creates unfavorable environment for pathogenic bacteria, LAB are also known to produce antimicrobial peptides (AMPs) these includes synthesizing bacteriocins, and releasing bioactive peptides from milk-based proteins such as milk lactoferrin fragments, and casein-derived peptides such as α s1-casein, α s2-casein, and β -casein all which can directly target closely related or Gram-negative bacteria. Bacteriocins specifically bulgaricin/bulgarican typically formed by *L. Bulgaricus* can cause direct cell membrane damage in pathogenic bacteria, the bacteriocins synthesized by *L. bulgaricus* function as cationic peptides that attach to the membrane surface of susceptible pathogenic bacteria, where they will induce pore formation thus disrupting membrane permeability. This disruption leads to the collapse of the proton motive force and the leakage of essential ions and small nutrient molecules, which will ultimately lead to the death of the pathogenic bacterial cell through a bactericidal mechanism. In addition, certain bacteriocins exhibit lytic activity by degrading the peptidoglycan layer of the bacterial cell wall, which results in cell lysis (Segura et al. 2016). Milk lactoferrin fragments are byproducts of the fermentation of milk proteins by LAB. These milk lactoferrin fragments inhibit bacterial growth via a similar mechanism with the bulgaricin, lactoferrin-derived fragments contain a plethora of cationic amino acids and possess amphipathic properties, this enabling them to interact directly with negatively charged molecules on microbial cell surfaces, including lipopolysaccharides (LPS) in Gram-negative bacteria and teichoic acids in Gram-positive bacteria (Paul & Somkuti. 2009). These interactions can help to disrupt membrane integrity by causing depolarization and leakage of cytoplasmic components, ultimately resulting in bacterial cell death without always causing cell lysis. Finally casein-derived peptides such as α s1-casein, α s2-casein which are also results of milk fermentation by LAB (Fan et al. 2019). These casein-derived peptides inhibit bacterial growth via the same amphipathic properties mechanism, not only that but certain peptides are able to interfere with bacterial metabolism using certain peptide fragments such as those derived from α s1-casein, which are able to enter bacterial cells without causing immediate membrane rupture and subsequently

disrupt intracellular activities, including protein synthesis and DNA replication (He et al. 2025). Furthermore, LAB exhibits competitive exclusion effects by rapidly utilizing available nutrients and occupying ecological niches, thereby limiting pathogen proliferation (De W Blackburn., & McClure, 2009).

The composition of each yogurt product may further explain the differences observed in inhibition zones. Yogurt X contained multiple probiotic strains, namely *L. acidophilus* and *Bifidobacterium* spp., in addition to the standard yogurt cultures. These bacteria are widely associated with antimicrobial activity and competitive exclusion against pathogens. However, yogurt X also contained relatively high amounts of fruit puree, nata de coco, sugar, and flavoring agents, which may provide additional nutrients that support bacterial growth thus reducing the net inhibitory effect. Yogurt Z similarly contained additional probiotic strains such as *L. paracasei* and *L. rhamnosus*, both of which are reported to possess antimicrobial and probiotic properties, however yogurt Z also uses preservatives such as potassium sorbate may have influenced LAB viability or metabolite production which potentially contributes to the slightly lower inhibition observed against *E. coli*. Yogurt Y, despite containing only the standard starter cultures, produced the largest inhibition zones against both pathogens. This may suggest that its formulation allowed optimal LAB activity and antimicrobial metabolite production despite lacking additional probiotic strains.

The synergistic activity of acidification, antimicrobial compound production, and competitive interactions explains the inhibition zones observed in this study and supports the potential role of yogurt-derived probiotics as a natural antimicrobial strategy against gastrointestinal pathogens.

Evaluation of Biofilm Disruption

The measurement of pathogen biofilm disruption by various yogurt was conducted by growing the biofilm in 96-well plates which is then incubated and added with the treatment then incubated for another 24 hours. The result is then quantified using the microplate reader. A few treatments were tested using this method which includes the addition of supernatant made from Yogurt X, Yogurt Y, and Yogurt Z, as well as chloramphenicol as the positive control and sterile aquadest as the negative control, to assess the effectivity of each yogurt and compare them with each other as well as the negative and positive control. The results of the analysis are shown in Table 2:

Table 2. Results of biofilm disruption test for *E. Coli* and *Salmonella* spp.

Strain	Percentage Change in Biofilm Biomass (%)				
	Yogurt			Control	
	X	Y	Z	+	-
<i>E. coli</i>	14.4±2.08 ^b	191.76±1.44 ^b	100.3±1.38 ^b	-72.26±1.27 ^c	100±1.25 ^a
<i>Salmonella</i> spp.	81.88±1.25 ^b	90.55±1.20 ^b	29.44±1.42 ^b	-33.07±1.61 ^c	100±1.32 ^a

The results show that when it comes to the treatment groups both *E. coli* and *Salmonella* spp. shows an increase of biofilm biomass as seen by the numbers on the table above. The highest increase in *E. coli* biofilm is the one where the treatment yogurt Y was added, with an increase of 191.76±1.44% in biofilm followed by yogurt Z with an increase of 100.3±1.38% in biofilm and lastly the lowest increase was seen in yogurt X with an increase of 14.4±2.08%. The bacteria *Salmonella* spp. also shows an increase in biofilm biomass with the yogurt Y addition yielding the highest result at 90.55±1.20%, followed by yogurt X with an increase of 81.88±1.25%, and finally the yogurt with the lowest increase seen in yogurt Z at 29.44±1.42%. The activity of the antibiotics on the other hand shows a different result where it can be seen that there is a decrease of biofilm biomass with the numbers being a decrease around 72.26±1.27% in *E. coli* and 33.07±1.61% in *Salmonella* spp.

The increase in biofilm biomass observed in the yogurt treatment groups is not in accordance with the theoretical expectations as LAB present in yogurt are known to produce antimicrobial metabolites such as lactic acid, hydrogen peroxide, biosurfactants, and bacteriocins that may interfere with biofilm formation and destabilize preformed biofilms (Tomé et al., 2023). These compounds are reported to inhibit bacterial adhesion, disrupt the extracellular polymeric substance (EPS) matrix, and reduce the metabolic activity of biofilm-associated bacteria. Not only that but certain milk-protein fermentation byproducts such as lactoferrin fragments are also known to disrupt preformed biofilm, milk lactoferrin fragments are able to disrupt bacterial biofilms through several mechanisms that affect both the biofilm structure and bacterial activity. After lactoferrin is broken down into active cationic peptides such as lactoferricin, these fragments can prevent bacteria from attaching to surfaces by inhibiting genes involved in the production of biofilm adhesins. In already formed biofilms, they destabilize the matrix by binding to and degrading extracellular DNA (eDNA), which helps maintain the biofilm structure, while also promoting bacterial movement that encourages biofilm dispersal. In addition, these fragments interfere with ATP-binding cassette transporters, increase the permeability of bacterial cell walls, and help break apart complex multi-species biofilms within a relatively short time (Ammons & Copié, 2013) Therefore based on all the theoretical background, the yogurt treatment was expected to decrease biofilm biomass rather than enhance it.

This abnormality in the result might be caused due to several factors. The first one being the possibility that the nutrient content of yogurt, including lactose, proteins, peptides, and other organic compounds, may have served as an additional nutrient source for the pathogenic bacteria. The availability of these nutrients could stimulate bacterial growth and EPS production, which leads to an increase in the biofilm biomass. Another possible reason is that the concentration of antimicrobial metabolites produced by LAB in the yogurt samples may not have been sufficient to effectively disrupt mature biofilms. Established biofilms are known to possess strong resistance mechanisms due to the protective EPS matrix, reduced metabolic activity of embedded cells, and limited penetration of antimicrobial compounds. As a result, the metabolites present in yogurt may have been unable to penetrate and disrupt the mature biofilm structure effectively within the treatment period used in this study. Meanwhile the reduction in biofilm biomass observed in the chloramphenicol treatment supports the validity of the experimental setup, as chloramphenicol is a broad-spectrum antibiotic capable of inhibiting bacterial protein synthesis, thereby suppressing bacterial growth and reducing biofilm development. However, the lower reduction observed in *Salmonella* spp. compared to *E. coli* may indicate species-specific differences in susceptibility to chloramphenicol or differences in biofilm structure and resistance mechanisms.

The differences in biofilm biomass observed among the yogurt treatments may also be caused by the distinct compositions and bacterial cultures present in each yogurt brand. Yogurt X contained multiple probiotic strains, namely *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium* spp. In addition, yogurt X contained a fruit component namely strawberry. The presence of additional probiotic strains may explain why yogurt X produced the lowest increase in *E. coli* biofilm biomass compared to the other yogurt treatments, as certain probiotic bacteria such as *L. acidophilus* and *Bifidobacterium* are known to produce antimicrobial compounds and compete with pathogenic bacteria for adhesion sites. However, despite this potential antibiofilm activity, the high sugar and fruit content in the yogurt may still have provided nutrients that supported bacterial growth and EPS production, resulting in an overall increase in biofilm biomass. Yogurt Y demonstrated the highest increase in biofilm biomass for both *E. coli* and *Salmonella* spp. This yogurt mainly

consisted of water, sugar, milk powder, stabilizers, and only contained the standard yogurt starter cultures *S. thermophilus* and *L. bulgaricus*. Unlike yogurt X and yogurt Z, yogurt Y did not contain additional probiotic strains with stronger documented antimicrobial or antibiofilm properties. Furthermore, the relatively high sugar content and limited fruit content may have created a nutrient-rich environment that enhanced bacterial growth and stimulated biofilm formation. The low concentration of strawberry extract (0.1%) also suggests that the product may contain minimal natural bioactive compounds capable of inhibiting bacterial activity. Meanwhile, yogurt Z showed moderate increases in biofilm biomass. This yogurt contained additional probiotic strains, namely *Lactobacillus paracasei* and *Lactobacillus rhamnosus*, which have been reported to exhibit antimicrobial and antibiofilm activity against several pathogenic bacteria. The presence of polydextrose may also function as a prebiotic compound that supports the growth of beneficial bacteria. Moreover, yogurt Z contained potassium sorbate, a preservative with antimicrobial properties, which may have contributed to the lower increase in *Salmonella* spp. biofilm biomass compared to yogurt X and yogurt Y. However, similar to the other yogurt samples, the presence of sugar, milk components, and stabilizers could still support bacterial growth, thereby reducing the

overall effectiveness of the yogurt against established biofilms.

These findings suggest that the antibiofilm activity of yogurt products is highly influenced by their composition which includes probiotic strain composition, sugar concentration, preservative content, and additional ingredients. Although probiotics and LAB are theoretically capable of inhibiting pathogenic biofilms, commercially available yogurt products may not contain sufficient concentrations of active antimicrobial metabolites to effectively disrupt mature biofilms under the conditions used in this study.

Evaluation of Biofilm Inhibition

The measurement of pathogen biofilm inhibition by various yogurt was conducted by inoculating the bacteria in 96-well plates along with the treatment which is then incubated for another 24 hours. The result is then stained with crystal violet and then quantified using the microplate reader. A few treatments were tested using this method which includes the addition of supernatant made from Yogurt X, Yogurt Y, and Yogurt Z, as well as chloramphenicol as the positive control and sterile aquadest as the negative control, to assess the effectivity of each yogurt and compare them with each other as well as the negative and positive control. The results of the analysis are seen in Table 3:

Table 3. Results of biofilm inhibition test for *E. Coli* and *Salmonella* spp.

Strain	Percentage Change in Biofilm Biomass (%)				
	Yogurt				Control
	X	Y	Z	+	-
<i>E. coli</i>	-25.14±1.25 ^b	-49.48±1.57 ^b	-32.56±1.46 ^b	-21.33±1.31 ^c	100±1.31 ^a
<i>Salmonella</i> spp.	-58.90±1.66 ^b	-52.59±1.67 ^b	-23.14±1.30 ^b	-9.67±1.21 ^c	100±1.46 ^a

From the results as seen above in table 3, the comparison of percentage change in biofilm biomass between 3 different yogurt brands and C+ Chloroamphenicol can be observed. The results show that there is a reduction of percentage in biofilm biomass in both *E. coli* and *Salmonella* spp. as seen by the numbers on the table above. The highest decrease of biofilm biomass can be seen in *E. coli* in which the treatment yogurt Y was added, with a decrease of 49.48±1.57% in biofilm followed by yogurt Z with a decrease of 32.56±1.46% in biofilm biomass and lastly the lowest decrease was seen in yogurt X with a decrease percentage of 25.14±1.25%. The bacteria *Salmonella* spp. also shows a decrease in biofilm biomass with the yogurt X addition yielding the highest result at 58.90±1.66%, followed by yogurt Y with an decrease of 52.59±1.67%, and finally the yogurt with the lowest decrease in biofilm biomass is seen in yogurt Z at 23.14±1.30%. The activity of the antibiotics shows a

similar result where it can be seen that there is a decrease of biofilm biomass with the numbers being a decrease around 21.33±1.31% in *E. coli* and 9.67±1.21% in *Salmonella* spp.

The decrease in biofilm biomass is in accordance with previous studies as it is known that Yogurt and the probiotic bacteria it contains are able to directly suppress pathogenic biofilm formation by interfering with bacterial attachment, modifying the surrounding microenvironment, and producing metabolites that compete with pathogenic microorganisms (Barzegari et al. 2020).

The biofilm inhibition activity observed in this study may be caused due to the metabolic products generated by LAB present in the yogurt samples. All three yogurt brands contained the starter cultures *S. thermophilus* and *L. bulgaricus*, while yogurt X also contained *L. acidophilus* and *Bifidobacterium* spp., and yogurt Z contains *L. paracasei* and *L. rhamnosus*. These probiotic

bacteria are known to produce organic acids, hydrogen peroxide, biosurfactants, bacteriocins, and other antimicrobial compounds that may interfere with bacterial adhesion and biofilm stability.

The first major mechanism in which biofilm formation is inhibited is the production of lactic acid and other organic acids which lowers the environmental pH which is known to reduce biofilm formation (Roy et al. 2021). A lower pH can inhibit the initial attachment of bacteria to surfaces, which is essential for the beginning of biofilm formation. When the surface pH of a biomaterial becomes unfavorable, bacteria are unable to attach firmly and tend to return to a planktonic, free-floating state (Jones, Cochrane, and Percival. 2014). A lower pH also alters the ionic properties of functional groups within the extracellular polymeric substance (EPS) matrix. This change decreases the surface charge density and reduces the number of negatively charged groups, such as carboxylate and phosphate groups, thereby weakening the electrostatic interactions needed to maintain the stability of the biofilm structure (Aguilera et al. 2008).

Besides producing organic acids that create unfavorable conditions for pathogenic bacteria to grow, LAB also produces AMPs including bacteriocins as well as bioactive peptides released from milk proteins during fermentation, such as lactoferrin fragments and casein-derived peptides including α s1-casein, α s2-casein, and β -casein, all of which can directly inhibit the activity of pathogenic bacteria. Bacteriocins such as bulgaricin produced by *L. bulgaricus* function as cationic peptides that interact with bacterial cell membranes, causing pore formation and leakage of intracellular components. This membrane disruption may lead to bacterial cell death and reduced viability of biofilm-associated cells (Segura et al., 2016). In addition to bacteriocins, LAB also produce milk protein-derived bioactive peptides which are generated during fermentation, including lactoferrin fragments which possess amphipathic properties that enable them to interact with negatively charged bacterial membranes and interfere with intracellular functions such as protein synthesis and DNA replication (Paul & Somkuti. 2009). And also casein-derived peptides which have the same mechanism (He et al., 2025). Not only that but LAB are also able to inhibit pathogenic bacteria activities via competitive exclusion by rapidly consuming available nutrients and occupying ecological niches, thereby restricting the growth and proliferation of pathogenic microorganisms (De W Blackburn & McClure, 2009).

The differences in the composition of each yogurt brand may further explain the variation in antibiofilm effectiveness observed between treatments. Yogurt Y demonstrated the strongest reduction in *E. coli* biofilm biomass despite containing only the standard starter cultures *S. thermophilus* and *L. bulgaricus*. Its relatively simple composition, consisting mainly of water, milk powder, and sugar, may have allowed more efficient

LAB metabolic activity and higher production of antimicrobial metabolites. Yogurt X on the other hand, although exhibits less effectiveness against *E. coli*, showed the highest reduction against *Salmonella* spp. This may be associated with the presence of additional probiotic strains such as *L. acidophilus* and *Bifidobacterium* spp., which are known to exhibit strong antagonistic effects against enteric pathogens through competitive exclusion and antimicrobial metabolite production (Servin, 2004). Meanwhile, yogurt Z showed moderate antibiofilm activity against *E. coli* and the lowest activity against *Salmonella* spp. Although yogurt Z contained additional probiotic strains including *L. paracasei* and *L. rhamnosus*, the formulation also included preservatives such as potassium sorbate and acidity regulators, which may have decreased LAB viability and metabolic activity.

An interesting finding from this test is the fact that the yogurt treatments demonstrated greater biofilm reduction compared to chloramphenicol. This result may be explained by the different mechanisms of action between antibiotics and probiotic-derived metabolites. Chloramphenicol is known to be bacteriostatic rather than bactericidal. It inhibits bacterial growth by blocking protein synthesis, but it does not immediately kill all bacterial cells. Some bacterial cells may still remain metabolically active enough to attach to surfaces and initiate early biofilm formation despite reduced growth rates which means that although overall bacterial proliferation is suppressed, residual attached cells and extracellular matrix production may still occur. In contrast to the chloroamphenicol, LAB metabolites may directly interfere with the physical process of biofilm establishment itself. Another important factor to consider is the fact that chloramphenicol is a single purified antimicrobial compound with one primary mechanism of action, whereas yogurt contains a complex mixture of metabolites and probiotic bacteria that may act synergistically. This multi-target effect can make LAB more effective in preventing surface attachment and EPS accumulation during early biofilm formation.

CONCLUSION

This study demonstrates that the commercial yogurt products sold in Indonesia exhibit the ability to inhibit the growth of *E. coli* and *Salmonella* spp, as well as to suppress biofilm formation, evidenced by a reduction in the amount of biofilm compared to the negative control in the test.

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