LenteraBio
p-ISSN: 2252-3979 e-ISSN: 2685-7871

Antibacterial Activity of *Coconut Yoghurt* against *Streptococcus mutans* in Vitro

Aktivitas Antibakteri Coconut Yoghurt terhadap Streptococcus mutans secara in Vitro

Ananda Amalia Isnaini*, Guntur Trimulyono
Biology Study Program, Faculty of Mathematic and Natural Sciences, Universitas Negeri Surabaya
*e-mail: anandaamalia.20029@mhs.unesa.ac.id

Abstract. *Streptococcus mutans* is one of the bacteria that causes dental caries. Treatment efforts by using antibiotics, but excessive use of antibiotics that do not comply with guidelines can cause resistance. *Coconut yoghurt (cocoghurt*) is a yoghurt product made from coconut milk fermented with the help of lactic acid bacteria and contains antibacterial compounds. This study aims to determine the antibacterial activity of *cocoghurt* made by varying the composition ratio of the bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (2:1, 1:1, and 1:2). The research was carried out in several stages, namely rejuvenation and making bacterial suspension, making coconut milk, making a *starter*, making *cocoghurt,* and antibacterial test used the well-diffusion method. The test results were analyzed using the *Kolmogorov-Smirnov* test, the *One-Way ANOVA* test, and the Duncan test. Based on the statistical analysis, *cocoghurt* made with variations in the composition of *starter* bacteria affected the growth of *S. mutans.* The treatment group showed a significant difference from the control group but did not indicate a significant difference from the other treatment groups. The best composition ratio of *starter* bacteria in making *cocoghurt* in inhibiting *S. mutans* is a ratio of 1:2 with an inhibitory zone diameter of 5.20 ± 1.72 mm. This research proves that *cocoghurt* can inhibit the growth of *S. mutans*, so expected it can be used as an alternative in the medication and prevention of dental caries.
Kata kunci: antibacterial activity; probiotics; clear zone; food security

Abstrak. *Streptococcus mutans merupakan salah satu bakteri penyebab karies gigi. Upaya pengobatan biasanya dilakukan dengan penggunaan antibiotik, tetapi penggunaan antibiotik yang tidak sesuai dengan pedoman dapat menimbulkan resistensi. Coconut yoghurt (cocoghurt) merupakan produk yoghurt berbahan baku santan kelapa yang difermentasi dengan bantuan bakteri asam laktat dan mengandung senyawa antibakteri. Penelitian ini bertujuan untuk mengetahui aktivitas antibakteri cocoghurt yang dibuat dengan variasi perbandingan komposisi bakteri Lactobacillus bulgaricus dan Streptococcus thermophilus (2:1, 1:1, dan 1:2). Penelitian dilakukan dengan beberapa tahapan, yaitu peremajaan dan pembuatan suspensi bakteri, pembuatan santan, pembuatan starter, pembuatan cocoghurt, dan uji antibakteri dengan metode difusi sumuran. Hasil uji dianalisis menggunakan uji Kolmogorov-Smirnov, uji One-Way ANOVA, dan uji Duncan. Berdasarkan hasil analisis statistik, cocoghurt yang dibuat dengan variasi perbandingan komposisi bakteri starter berpengaruh terhadap pertumbuhan S. mutans. Kelompok perlakuan menunjukkan beda nyata terhadap kelompok kontrol, tetapi tidak menunjukkan beda nyata terhadap kelompok perlakuan lainnya. Perbandingan komposisi terbaik bakteri starter pada pembuatan cocoghurt dalam menghambat S. mutans adalah perbandingan 1:2 dengan diameter zona hambat sebesar 5,20 ± 1,72 mm. Penelitian ini membuktikan bahwa cocoghurt mampu menghambat pertumbuhan S. mutans, sehingga diharapkan dapat digunakan sebagai alternatif dalam pengobatan dan pencegahan karies gigi.*
Kata kunci: *aktivitas antibakteri; probiotik; zona hambat; ketahanan pangan*

INTRODUCTION

Dental caries is a condition of teeth that are damaged due to lack of oral hygiene, causing plaque buildup to damage hard tooth tissue (Novita, 2016). Plaque is a thin layer that contains a group of bacteria and forms in the mouth by adhering to the surface of the teeth and can form acid when united with sugar in food (Munadirah and Saleh, 2019). *Streptococcus mutans* is the primary cause of dental caries (Putri *et al*., 2017). The bacteria can adhere to the tooth surface (cariogenic) and are aciduric and acidogenic (Rahman *et al*., 2017).

In developed and developing countries, the percentage of dental caries problems in children reaches more than 80% (Suanda, 2018). According to the results of Riskesdas in 2018, the percentage of people with dental caries in Indonesian society was 88.8% with a prevalence in the group of children aged 5-9 years of 92.6% and a prevalence in the group of children aged 10-14 years of 73.4% (Ministry of Health RI, 2018). Meanwhile, according to the World Health Organization (2017) in the 2015 Global Burden of Disease Study, permanent tooth decay is ranked first with a total of 2.3 billion people, while in children it is ranked 12th with a total of 560 million children.

Efforts that can be made for the treatment and prevention of dental caries are the use of antibiotics or antimicrobial agents (Qiu *et al*., 2020). The use of antibiotics must be optimized by prescribing drugs according to guidelines, so that they can provide good results for everyone, especially for the most vulnerable groups (Thompson *et al*., 2021). However, long-term use of antibiotics can lead to adverse effects, namely gastrointestinal disorders to fatal anaphylactic shock (severe allergic reaction) and the development of antibacterial resistance (Oberoi *et al*., 2015).

Another alternative that can be used to treat dental caries is the use of probiotics (Nugraha *et al*., 2021). One of the probiotic drinks known to the public is yogurt (Pereira and Rodrigues, 2018). Yoghurt is generally made using cow's milk (Purwantiningsih *et al*., 2022). However, with the problem of intolerance and allergies and the desire to eat vegetarian food, the demand for non-dairy probiotics has increased (Zhi *et al*., 2021). The main ingredient that can be used as a substitute for cow's milk in making yogurt is coconut milk (Yunita *et al*., 2023). Coconut milk yogurt is known as *cocoghurt* or *niyoghurt* (Sarah *et al*., 2021).

Coconut yogurt (*cocoghurt*) is a yoghurt product made from coconut milk that is fermented with the help of Lactic Acid Bacteria (LAB) (Destiana *et al*., 2021). The best combination of two LAB to be utilized in making yoghurt is *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, because both can work well together during the fermentation process (Hendarto *et al*., 2019). In addition, LAB also acts in inhibiting the growth of other microbes, such as pathogenic bacteria because it can produce lactic acid, hydrogen peroxide (H_2O_2), and bacteriocins (Hamidah *et al*., 2019).

According to Pato *et al*. (2019), *cocoghurt* has the potential to have therapeutic benefits due to the content of medium chain fatty acids derived from coconut milk, namely lauric acid which provides various health benefits, including increasing immunity and as an antimicrobial agent. Based on research by Anzaku *et al*. (2017), lauric acid is able to inhibit several clinical isolates, namely *Escherichia coli*, *Mycobacterium tuberculosis*, *Salmonella* spp., *Staphylococcus aureus*, and *Streptococcus pneumonia*. In a previous study conducted by Qadi *et al*. (2023), showed that the length of fermentation and cold storage of coconut milk fermented with *Lactiplantibacillus plantarum* can affect physicochemical characteristics, shelf life, proximate and chemical composition, antioxidant activity, and antibacterial activity tested on bacteria *S. aureus*, *Bacillus subtilus*, *E. coli*, *Cronobacter sakazakii*, *Bacillus cereus*, and *Salmonella typhi*. Thus, this study was conducted to test the antibacterial activity of *cocoghurt* in inhibiting the growth of *S. mutans* bacteria.

MATERIAL AND METHODS

The research was conducted in October 2023-January 2024 at the Microbiology Laboratory and the Genetics and Molecular Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya.

In this study, the tools used consisted of petri dishes, test tubes, test tube racks, stirring rods, beaker glass, erlenmeyer, mortar and pestle, spirtus lamp, ose needle, micropipette, syringe, cork borer diameter 6 mm, magnetic stirrer, ruler, scales, analytical balance, hot plate, vortex, pH meter, stove, pot, spoon, glass bottle, filter cloth, incubator, autoclave, UV-Vis spectrophotometer, and LAF (*Laminar Air Flow*). Meanwhile, the materials used included coconut fruit meat, bacterial cultures of *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *S. thermophilus*, *S. mutans* (obtained from the Microbiology Laboratory, Universitas Brawijaya), skim milk, sugar, water, de Man Ragosa Sharpe Agar (MRSA) medium [Merck cat no. 1.10660.05000], de Man Ragosa Sharpe Broth (MRSB) medium [Himedia cat no. M369-500G], Nutrient Agar (NA) medium [Merck cat no. 1.05450.0500], *Ciprofloxacin* antibiotic, distilled water, NaCl 0.9%, 70% alcohol, cotton swab, blue tip, yellow tip, and aluminum foil.

MRSA media was made by dissolving 6.82 grams of media in 100 mL of distilled water, MRSB media was made by dissolving 8.27 grams of media in 150 mL of distilled water, and NA media was made by dissolving 10 grams of media in 500 mL of distilled water. After that, all three were put in different beaker glasses and heated using a hot plate and stirred until homogeneous. The media was then poured into an erlenmeyer and covered with cotton, then sterilized using an autoclave at 121 ^{o}C and 1 atm pressure for 15 minutes (Zuhri, 2019).

Rejuvenation of *starter* bacteria was carried out by taking a culture of *L. bulgaricus* and *S. thermophilus* bacteria each as much as one ose and then cultured on different slanted MRSA media and incubated at 37°C for 24 hours (Sherlahwaty and Syarmalina, 2015). Furthermore, each bacterium was made into suspension by taking one ose and then inoculated into 20 mL of different MRSB media and incubated at 37°C for 24 hours (Alrosyidi *et al*., 2015).

Making coconut milk was done by weighing 500 grams of grated coconut meat, then adding 1000 mL of hot water at 60°C (1:2 ratio), then squeezed using a filter cloth and collected in a container (Raharjanti *et al*., 2019).

Starter preparation was carried out by referring to the research of Raharjanti *et al*. (2019), by making each 100 mL skim milk pasteurized for 10 minutes at 80°C. Next, each was inoculated with *L. bulgaricus* and *S. thermophilus* bacteria as much as 2% (v/v) for different skim milk mediums that had been allowed to stand until the temperature reached 43-45°C then incubated for 24 hours at 37°C (F1 *starter*). After that, continue making *starter* F2 by psteurizing as much as 50 mL skim milk and 50 mL coconut milk that has been homogenized at 80°C for 10 minutes. Furthermore, each F1 *starter* was inoculated as much as 5% (v/v) on different mediums that had been allowed to cool down until the temperature reached 43-45°C and then incubated for 24 hours at 37°C. Thus, *starter* F2 can be used in making *cocoghurt*.

The stages of making *cocoghurt* include mixing *L. bulgaricus* and *S. thermophilus* bacterial starters in a ratio of 2:1, 1:1 and 1:2 and then stirring gently (Handayani, 2021). Coconut milk was prepared as much as 750 mL and then poured into 3 different glass bottles with 250 mL in each bottle, then each bottle was given 15% (b/v) skim milk powder and 5% (b/v) sugar and stirred until homogeneous. Next, the coconut milk was pasteurized for 10 minutes at 80°C and cooled to 45°C, then inoculated with 5% (v/v) *starter* and incubated for 6 hours at 37°C (Raharjanti *et al*., 2019).

Rejuvenation of *S. mutans* bacteria is carried out by taking one ose of bacterial culture using a sterilized ose needle and then scratching it on an inclined NA medium aseptically, then incubating it in an incubator at 37°C for 24 hours (Annisa and Mursyid, 2020). After that, a suspension was made by taking one ose and then inoculated into 10 mL of 0.9% NaCl solution (Sari *et al*., 2019). Furthermore, a UV-Vis spectrophotometer at a wavelength of 625 nm was used in measuring the turbidity of the suspension by adjusting the absorbance value to reach 0.08-0.1 or equivalent to 1.5×10^8 CFU/mL (0.5 Mc Farland standard) (Djunaidy *et al*., 2020).

The pH test was carried out using a pH meter. Before use, the pH meter is calibrated with pH 4 and pH 7 *buffers* and then washed with distilled water and cleaned using a tissue. The pH meter can then be used to measure the pH of the sample and each time it will repeat or measure the pH of other samples the *probe* is rinsed using distilled water and then cleaned with a tissue (Masykur and Kusnadi, 2015)

The antibacterial activity test was carried out by the well diffusion method with the pour plate technique. A total of 1 mL of suspension was poured into a sterile Petri dish and then given 15 mL of NA media, homogenized, and allowed to solidify (Sarifuddin, 2022). After that, make a number of 3 wells with a 6 mm cork borer and each well is filled with *cocoghurt* made with variations in the composition of *L. bulgaricus* and *S. thermophilus* bacteria (2:1, 1:1, and 1:2), positive control, and negative control of 50 µl and then incubated for 24 hours at 37°C (Fitrianarni *et al*., 2014). Each treatment was done with 5 repetitions. Measurement of the diameter of the inhibition zone was carried out using a ruler with reference to the following formula (Harti, 2015):

$$D = \frac{(D1 - d1) + (D2 - d2)}{2}$$

Description:
D: Diameter of zone of inhibition
D1: Diameter of horizontal zone of inhibition
d1: Diameter of horizontal well
D2: Diameter of vertical zone of inhibition
d2: Diameter of vertical pits

The antibacterial test data were statistically analyzed using *Statistical Product and Service Solution* (SPSS) 23.0 *for windows software*, including *Kolmogorov-Smirnov* test, *One-Way ANOVA* test, and Duncan test.

RESULTS

Research on the antibacterial activity of *cocoghurt* against *S. mutans* showed that *cocoghurt* made with variations in the composition of *L. bulgaricus* and *S. thermophilus* bacteria (Lb: St) was able to inhibit the growth of *S. mutans*. This can be seen through the formation of an inhibition zone around the well (Figure 1).

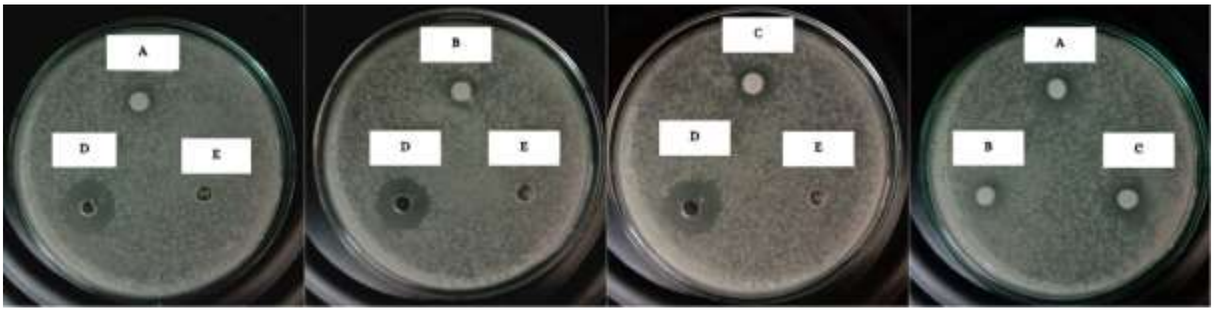

Figure 1. Results of *cocoghurt* antibacterial activity test against *S. mutans* in vitro; (A) Lb: St (2:1); (B) Lb: St (1:1); (C) Lb: St (1:2); (D) Positive control (*Ciprofloxacin* 0.5%); (E) Negative control (distilled water)

Data regarding the diameter of the inhibition zone that has been obtained is analyzed using *Statistical Product and Service Solution* (SPSS) 23.0 *for windows software*. The *Kolmogorov-Smirnov* test results showed Sig. value of 0.150 which means the Sig value> (α) 0.05 or defined as normally distributed data. After that, the *One-Way ANOVA* test was conducted to determine the impact/influence of each treatment on *S. mutans* bacteria. The results of the *One-Way ANOVA* test showed Sig. value of 0.00 which means the Sig value < (α) 0.05 or defined there is an effect of giving *cocoghurt* made with variations in the ratio of bacterial composition (Lb: St) on *S. mutans* bacteria. Furthermore, Duncan's test was conducted to compare the results of each treatment (Table 1).

Table 1. Average diameter of inhibition zone of *cocoghurt* antibacterial activity test results against *S. mutans*

Treatment	Mean Inhibition Zone Diameter (mm) ± SD*
Negative control (distilled water)	0.00 ± 0.00^{a}
Lb: St (2:1)	3.40 ± 1.39^{b}
Lb: St (1:1)	$4.50 \pm 1.12^{b,c}$
Lb: St (1:2)	5.20 ± 1.72^{c}
Positive control (*Ciprofloxacin* 0,5%)	12.0 ± 0.50^{d}

*)different notations indicate significant differences based on Duncan's test with a significance level (α) of 0.05. Lb: *Lactobacillus bulgaricus*. St.: *Streptococcus thermophillus*

The Duncan test results showed that the control group was significantly different from the treatment groups Lb: St (2:1), Lb: St (1:1), and Lb: St (1:2). Meanwhile, the treatment of Lb: St (1:1) treatment was not significantly different from the Lb: St (2:1) and Lb: St (1:2).

Cocoghurt made with variations in the ratio of *L. bulgaricus* and *S. thermophilus* (Lb: St) bacteria composition had similar characteristics, namely thick viscosity, typical yoghurt aroma, white color, and sweet and sour taste. However, the sour taste produced by the three *cocoghurt* has different levels. This can be seen through the average pH value of the *cocoghurt* produced (Figure 2).

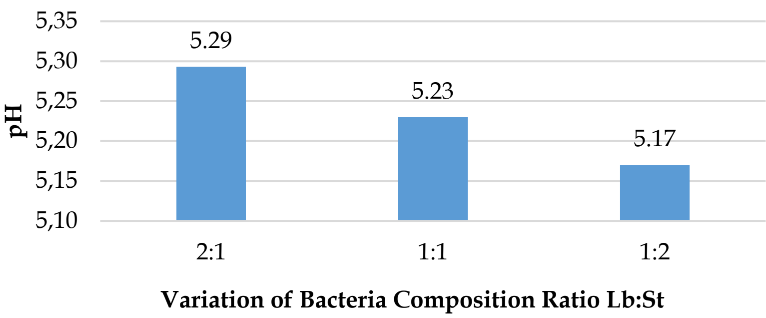

Figure 2. Average pH value of *cocoghurt* made with variations in bacterial composition ratio (Lb: St)

DISCUSSION

Based on the results of statistical tests, it shows that *cocoghurt* made with variations in the ratio of bacterial composition (Lb: St) has an effect in inhibiting the growth of *S. mutans*. The treatment group showed a significant difference against the control group, but did not show a significant difference against the other treatment groups which was characterized by the similarity of notations between *cocoghurt* made with a ratio of bacterial composition (Lb: St) 1:1 with 2:1 and 1:1 with 1:2. This indicates that each *cocoghurt* has a similar ability to inhibit the growth of *S. mutans*. *Streptococcus mutans* is known to produce acid (acidogenic) and live in an acidic environment (asiduric) (Suwito *et al*., 2017). In addition, these bacteria can grow at a pH ranging from 5.2-7 at temperatures around 18-40°C (Fatmawati, 2015). Thus, when *S. mutans* interacts with *cocoghurt*, which has a pH range of 5.1-5.3, it produces less than maximum inhibition, because it is suspected that *S. mutans* can still grow because both can produce acid.

In antibacterial tests, the use of the well method can make it easier to measure the size of the inhibition zone, because bacteria do not only move on the top surface of the media, but also on the bottom surface of the media (Nurhayati *et al*., 2020). The results of the antibacterial test in this study showed that *cocoghurt* made with variations in the composition of Lb: St was able to inhibit the growth of *S. mutans* bacteria as evidenced by the formation of an inhibition zone around the well. Based on the results of research by Pato *et al*. (2019), *cocoghurt* is known to contain medium chain fatty acids, one of which is lauric acid. Lauric acid is a natural fatty acid that has antibacterial activity (Casillas-Vargas *et al*., 2021). In connection with this, it is suspected that the content of lauric acid in *cocoghurt* plays a role in the formation of inhibition zones in this study. The mechanism of lauric acid as an antibacterial is by damaging the bacterial cell wall, so that it can cause a decrease in activity and disruption of the metabolism of bacterial cell growth, and can even result in cell death (Nitbani *et al*., 2022). In addition to lauric acid, *cocoghurt* also contains lactic acid bacteria (LAB) which also play a role in producing antibacterial compounds. According to Bharti *et al*. (2015), antibacterial compounds produced by LAB are organic acids, especially lactic acid, hydrogen peroxide (H_2O_2), and bacteriocins.

Lactic acid plays a role in lowering the pH so that yogurt can be durable because pathogenic bacteria cannot grow in an acidic environment (Jannah *et al*., 2014). According to Kasi *et al*. (2017), the process of inhibiting pathogenic bacteria by lactic acid is by disrupting the integrity of the bacterial cell membrane through the diffusion of lactic acid into the growth medium, so that these conditions cause the absorption of nutrients needed by bacteria to grow and cause their growth to be inhibited because the metabolic process does not run. Meanwhile, H_2O_2 is a strong oxidizer that can cause an increase in membrane permeability through the mechanism of cell membrane fatty peroxidase and denaturation of several enzymes (Tian *et al*., 2018). Bacteriocins are also compounds that have the ability to inhibit microbial growth (Dewi *et al*., 2019). In inhibiting the growth of Gram-positive bacteria, bacteriocins prevent the synthesis of peptidoglycan, resulting in cell wall weakness and bacterial cell rupture (Hamidah *et al*., 2019).

Cocoghurt made with a combination of *L. bulgaricus* (Lb) and *S. thermophilus* (St) bacteria has characteristics of thick viscosity, typical yogurt aroma, white color, and sweet and sour taste. This is in line with the research of Pato *et al*. (2021) which showed that *cocoghurt* has a thick viscosity because the pH of the medium decreases and as a result protein clumping occurs, a dominant distinctive aroma caused by pure coconut milk, a white color resulting from coconut milk and skim milk, a sweet taste resulting from the addition of sucrose to the fermentation medium, and a sour taste caused by the formation of lactic acid during the fermentation process. Meanwhile, the difference in acidity levels in the three *cocoghurt* was caused by differences in the ratio of the composition of Lb: St bacteria used. The difference in the composition ratio affects the process of *cocoghurt* fermentation, so it also affects the pH value produced.

In making *cocoghurt*, the ingredients used are coconut milk and skim milk. Unfermented coconut milk has a pH value ranging from 5.6-6.3 (Alyaqoubi *et al*., 2015). Meanwhile, skim milk has a neutral pH, which is 7 (Handayani *et al*., 2014). The average pH value produced by *cocoghurt* after fermentation is 5.1-5.3. The decrease in pH value is influenced by the results of LAB fermentation in the form of lactic acid obtained from converting simple sugars into organic acids (Ningtyas *et al*., 2017). According to Sieuwerts (2016), during the fermentation process, the first exponential phase is characterized by the growth of *S. thermophilus* at a pH close to neutral, so it is more effective in absorbing amino acids than *L. bulgaricus*. Meanwhile, *L. bulgaricus* is known to be more acid tolerant than *S. thermophilus* (Mena and Aryana, 2020). *Lactobacillus bulgaricus* grows optimally with pH conditions ranging from 4-5.5 at a temperature of 34-45°C (Anggraini and Ardyati, 2017).

According to Wirawati and Nirmagustina (2022), a decrease in pH due to the accumulation of lactic acid also has an effect in inhibiting the growth of several species of pathogenic bacteria. The mechanism of pH in inhibiting the growth of pathogenic bacteria is by means of organic acids in an undecomposed form entering through the cell membrane and the pH in the cell will decrease and the decomposition of acid molecules (release of protons and anions), so that the accumulation of acids in an undecomposed form will make the permeability of the cell membrane change and cause the material transport system in bacteria to be destroyed and the cell will experience lysis (Halim *et al*., 2013).

In inhibiting *S. mutans*, the average pH value of *cocoghurt* made with a ratio of bacterial composition (Lb: St) 1:2 produces a larger average diameter of the inhibition zone than the average pH value of *cocoghurt* made with a ratio of bacterial composition (Lb: St) 2:1 and 1:1. This is because *S. thermophilus* has a higher acidification rate than other LAB (Rahmayani *et al*., 2021). In addition, the high acid produced by *cocoghurt* made with a ratio of bacterial composition (Lb: St) 1:2 is also thought to be caused by more CO_2 and formic acid produced by *S. thermophilus* to support the growth of *L. bulgaricus* in producing lactic acid. As according to Sansawal *et al.* (2017) which shows that the fermentation mechanism of making yogurt generally begins with the growth of *S. thermophilus* which releases CO_2 from the breakdown of urea and formic acid, then *S. thermophilus* consumes O_2 contained in the medium, thus causing oxidation-reduction potential that can support the growth of *L. bulgaricus* because it is more tolerant of acid. Furthermore, *L. bulgaricus* will hydrolyze milk proteins and produce amino acids and nonpolar bioactive peptides to support the growth of *S. thermophilus* which will occur again (Miskiyah *et al*., 2020). *Lactobacillus bulgaricus* also plays a role in breaking down lactose into lactic acid ($C_3H_6O_3$) which will easily dissociate into H^+ and $CH_3CHOHCOO^-$ ions (Rahman *et al*., 2019). The more H^+ ions that accumulate, the more acid is produced, so that the resulting pH value also decreases (Rasbawati *et al*., 2019). Therefore, it can be seen that the lower the pH value, the wider the inhibition zone produced (Afriani, 2017). This is because pH is closely related to enzyme activity that is influential in bacterial growth, namely to catalyze reactions related to bacterial growth (Fajar *et al*. 2022).

The diameter of the inhibition zone of *cocoghurt* was smaller than the positive control *Ciprofloxacin*. This indicates that the inhibition of *Ciprofloxacin* positive control against *S. mutans* growth is stronger than the inhibition of *cocoghurt*. *Ciprofloxacin* is known to be classified as a broad-spectrum antibiotic that can fight Gram-negative and Gram-positive bacteria by inhibiting DNA synthesis and enzyme inactivation (Govindaraju *et al*., 2021). In this case, inhibition of bacterial DNA synthesis occurs due to interference by *Ciprofloxacin* antibiotics with the enzyme DNA topoisomerase or commonly called DNA-gyrase so that it will have an effect as an antibacterial (Sari *et al*., 2019). Meanwhile, *S. mutans* is classified as a Gram-positive bacterium that causes dental caries (Tandra *et al*., 2022). Gram-positive bacteria are composed of a thicker peptidoglycan layer with a range between 20-80 nm, so that when the bacterial cell wall interacts with antibacterial compounds, the size of the inhibition produced around the well will be smaller because the cell wall is difficult to destroy (Sukmiwati *et al*., 2018).

In antibacterial tests, the difference in inhibition zones produced is influenced by several factors, such as pH, culture medium, incubation conditions, organism sensitivity, microbial type, and agar diffusion speed (Yusriyani *et al*., 2023). Meanwhile, agar diffusion speed is influenced by microbial concentration, media composition, incubation temperature, and incubation duration (Maharani *et al*., 2023). Therefore, it can be known that the wider the diameter of the inhibition zone, the stronger the inhibition power produced (Fatonah *et al*., 2022).

Making yogurt with coconut milk as the basic ingredient is one of the results of product diversification that is rich in benefits. *Cocoghurt* can be used as an alternative for people with lactose intolerance and other milk allergies in consuming yogurt (Peters *et al*., 2023). According to Pato *et al.* (2019) the content of probiotic bacteria and lauric acid in *cocoghurt* can be beneficial for human health. Therefore, the content of antibacterial compounds in *cocoghurt* has the potential to be used as a treatment and prevention of dental caries, so as to reduce the impact of antibiotic resistance in overcoming dental caries.

CONCLUSION

Based on research on the antibacterial activity of *cocoghurt* against *S. mutans*, it shows that *cocoghurt* has antibacterial activity against *S. mutans*. The best composition ratio of *L. bulgaricus* and *S.*

thermophilus bacteria in making *cocoghurt* in inhibiting the growth of *S. mutans* is a ratio of 1:2 with an inhibition zone of 5.20 ± 1.72 mm.

REFERENCES

Afriani N, Yusmarini Y, dan Pato U, 2017. Aktivitas Antimikroba *Lactobacillus plantarum* 1 yang Diisolasi dari Industri Pengolahan Pati Sagu terhadap Bakteri Patogen *Escherichia coli* FNCC-19 dan *Staphylococcus aureus* FNCC-15. *JOM FAPERTA*; 4(2): 1-12.

Anggraini AA dan Ardyati T, 2017. Pengaruh Kombinasi Starter Bakteri Asam Laktat (BAL) pada Pembuatan Keju Kedelai (Soy Cheese). *Biotropika: Journal of Tropical Biology*; 5(3): 83-85.

Alrosyidi AF, Darmawati A, Isnaeni I, dan Sugijanto NEN, 2015. Aktivitas Antibakteri Kombinasi Susu Probiotik *Lactobacillus bulgaricus* dan *Streptococcus thermophilus* terhadap Bakteri Penyebab Diare. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*; 2(2): 44-47.

Alyaqoubi S, Abdullah A, Samudi M, Abdullah N, Addai ZR, dan Musa KH, 2015. Study of Antioxidant Activity and Physicochemical Properties of Coconut Milk (Pati Santan) in Malaysia. *Journal of Chemical and Pharmaceutical Research*; 7(4): 967-973.

Annisa RN dan Mursyid M, 2020. Efektivitas Antimikroba Minyak Zaitun sebagai Bahan Tambahan Pasta Gigi terhadap Bakteri *Streptococcus mutans*. *BIOMA: Jurnal Biologi dan Pembelajarannya*; 2(2): 1-8.

Anzaku AA, Akyala JI, Juliet A, dan Obianuju EC, 2017. Antibacterial Activity of Lauric Acid on Some Selected Clinical Isolates. *Ann. Clin. Lab. Res*; 5(2): 1-5.

Bharti V, Mehta A, Singh S, Jain N, Ahirwal L, dan Mehta S, 2015. Bacteriocin: A Novel Approach for Preservation of Food. *International Journal of Pharmacy and Pharmaceutical Sciences*; 7(9): 20-29.

Casillas-Vargas G, Ocasio-Malavé C, Medina S, Morales-Guzmán C, Del Valle RG, Carballeira NM, dan Sanabria-Ríos DJ, 2021. Antibacterial Fatty Acids: An Update of Possible Mechanisms of Action and Implications in The Development of The Next-Generation of Antibacterial Agents. *Progress in lipid research*; 82: 101093.

Destiana ID, Aprilia D, dan Hermalia S. 2021. Karakteristik Mutu Kimia dan Biologi *Cocoghurt* dengan Perbedaan Konsentrasi Pektin dan Pisang sebagai Prebiotik Alami. *EDUFORTECH*; 6(2): 134-138.

Dewi LF, Sartini S, dan Rahmiati R, 2019. Isolasi Bakteri Asam Laktat dari Usus Sapi (*Bos taurus*) serta Kemampuannya dalam Menghambat Pertumbuhan Bakteri *Eschericia coli* dan *Shigella sp*. *Jurnal Ilmiah Biologi UMA (JIBIOMA)*; 1(1):21–27.

Djunaidy VP, Putri DKT, dan Setyawardhana RHD, 2020. Pengaruh Kitosan Sisik Ikan Haruan (*Channa striata*) terhadap Jumlah Koloni Interaksi *Streptococcus sanguinis* dan *Streptococcus mutans* secara *In Vitro*. *Dentin*; 4(3): 100-110.

Fajar I, Perwira IY, dan Ernawati NM, 2022. Pengaruh Derajat Keasaman (pH) terhadap Pertumbuhan Bakteri Toleran Kromium Heksavalen dari Sedimen Mangrove di Muara Tukad Mati, Bali. *Current Trends in Aquatic Science*; 1: 1-6.

Fatmawati DWA, 2015. The Relationship between *Streptococcus Mutans* Biofilms and the Risk of Dental Caries. Stomatognathic- *Journal of Dentistry*. ISSN 2442-4935.

Fatonah NS, Pertiwi FD, Rezaldi F, Abdillah NA, Dita A L, dan Fadillah MF, 2022. Uji Aktivitas Antibakteri *Escherichia Coli* pada Formulasi Sediaan Sabun Cair Mandi Probiotik dengan Metode Bioteknologi Fermentasi Kombucha Bunga Telang (*Clitoria ternatea* L). *AGRIBIOS*; 20(1): 27-37.

Fitrianarni D, Ibrahim M, dan Trimulyono G, 2014. Aktivitas Antibakteri Yoghurt Susu Sapi dan Yoghurt Susu Kedelai terhadap *Shigella dysenteriae* secara in Vitro. *LenteraBio*; 3(1): 97-102.

Govindaraju L, Jenarthanan S, Subramanyam D, dan Ajitha P, 2021. Antibacterial Activity of Various Intracanal Medicament Against *Enterococcus faecalis*, *Streptococcus mutans*, and *Staphylococcus aureus*: An in Vitro Study. *Journal of Pharmacy & Bioallied Sciences*; 13(Suppl 1): S157.

Halim CN dan Zubaidah E, 2013. Studi Kemampuan Probiotik Isolat Bakteri Asam Laktat Penghasil Eksopolisakarida Tinggi Asal Sawi Asin (*Brassica juncea*). *Jurnal Pangan dan Agroindustri*, 1(1): 129–137.

Hamidah MN, Rianingsih L, dan Romadhon R, 2019. Aktivitas Antibakteri Isolat Bakteri Asam Laktat dari Peda dengan Jenis Ikan Berbeda terhadap *E. coli* dan *S. aureus*. *Jurnal Ilmu dan Teknologi Perikanan*; 1(2): 11-21.

Handayani GN dan Ida N, 2014. Pemanfaatan Susu Skim sebagai Bahan Dasar dalam Pembuatan Produk Olahan Makanan Tradisional Dangke Dengan Bantuan Bakteri Asam Laktat. *Jurnal farmasi UIN Alauddin Makassar*; 2(2): 56-61.

Handayani KR, 2021. Pengaruh Komposisi Bakteri pada Yoghurt Difortifikasi Buah Jamblang (*Syzygium cumini* L.) terhadap Karakteristik Organoleptik dan Tingkat Kesukaan Yoghurt. *Jurnal Ilmiah Pharmacy*; 8(1): 27-35.

Harti, A.S. 2015. Mikrobiologi Kesehatan. Yogyakarta: CV Andi.

Hendarto DR, Handayani AP, Esterelita E, dan Handoko YA, 2019. Mekanisme Biokimiawi dan Optimalisasi *Lactobacillus bulgaricus* dan *Streptococcus thermophilus* dalam Pengolahan Yoghurt yang Berkualitas. *J. Sains Dasar*; 8(1): 13-19.

Jannah AM, Legowo AM, Pramono YB, AlBaarri AN, dan Abduh SBM, 2014. Total Bakteri Asam Laktat, pH, Keasaman, Citarasa, dan Kesukaan Yogurt Drink dengan Penambahan Ekstrak Buah Belimbing. *Jurnal Aplikasi Teknologi Pangan*, 3(2): 7-11.

Kasi PD, Ariandi A, dan Mutmainnah H, 2017. Uji Antibakteri Isolat Bakteri Asam Laktat yang Diisolasi dari Limbah Cair Sagu terhadap Bakteri Patogen. *Biotropika: Journal of Tropical Biology*, 5(3): 97-101.
Kementerian Kesehatan RI, 2018. *Hasil Utama Riskesdas 2018*. Jakarta: Badan Penelitian dan Pengembangan Kesehatan.
Maharani D, Rafika R, Hasan ZA, dan Artati A, 2023. Pengaruh Replikasi Pemanasan Media Nutrient Agar terhadap Nutrisi Media, pH Media dan Jumlah Koloni Bakteri. *Prosiding Asosiasi Institusi Pendidikan Tinggi Teknologi Laboratorium Medik Indonesia*, 2: 73-85.
Masykur A dan Kusnadi J, 2015. Karakteristik Kimia dan Mikrobiologi Yoghurt Bubuk Kacang Tunggak (*Vigna unguiculata* L.) Metode Pengeringan Beku (Kajian Penambahan Starter dan Desktrin). *Jurnal Pangan dan Agroindustri*; 3(3): 1171-1179.
Mena B dan Aryana K, 2020. Effect of Lactose on Acid Tolerance of Yogurt Culture Bacteria. *Food and Nutrition Sciences*; 11: 457-462.
Miskiyah N, Juniawati N, dan Yuanita L, 2020. Mutu Starter Kering Yoghurt Probiotik di Berbagai Suhu Selama Penyimpanan. *Jurnal Penelitian Pascapanen Pertanian*; 17(1): 15-23.
Munadirah M dan Saleh M, 2019. Efektifitas Metode Sikat Gigi Vertikal dan Horizontal terhadap Penurunan Indeks Plak pada Anak Usia 9-11 Tahun di Mi DDI Seppange di Desa Tungke Kecamatan Bengo Kabupaten Bone. *Media Kesehatan Gigi: Politeknik Kesehatan Makassar*; 18(1): 30-33.
Ningtyas JC, Ramadhan AM, dan Rijai L, 2017. Karakteristik dan Aktivitas Antibakteri Yoghurt Sari Buah Sirsak (*Annona muricata* L.) terhadap Bakteri Flora Usus. *Proceeding of Mulawarman Pharmaceuticals Conferences*; 5(1), 149–156.
Nitbani FO, Tjitda PJP, Nitti F, Jumina J, dan Detha AIR, 2022. Antimicrobial Properties of Lauric Acid and Monolaurin in Virgin Coconut Oil: a review. *ChemBioEng Reviews*; 9(5): 442-461.
Novita W, 2016. Uji Aktivitas Antibakteri Fraksi Daun Sirih (*Piper Betle* L) terhadap Pertumbuhan Bakteri *Streptococcus Mutans* Secara in Vitro. *Jambi Medical Journal*; 4(2): 140-155.
Nugraha DH, Fatikhah N, Heriyanto Y, dan Chaerudin DR, 2021. Efektivitas Berkumur Larutan Probiotik terhadap Peningkatan pH Saliva (Studi Literatur). *Jurnal Kesehatan Siliwangi*; 2(1): 389-393.
Nurhayati LS, Yahdiyani N, dan Hidayatulloh A, 2020. Perbandingan Pengujian Aktivitas Antibakteri Starter Yoghurt dengan Metode Difusi Sumuran dan Metode Difusi Cakram. *Jurnal Teknologi Hasil Peternakan*; 1(2): 41-46.
Oberoi SS, Dhingra C, Sharma G, dan Sardana D, 2015. Antibiotics in Dental Practice: How Justified Are We. *International Dental Journal*; 65(1): 4-10.
Pato U, Yusuf Y, Panggabean IP, Handayani NP, Adawiyah N, dan Kusuma AN, 2019. Influence of Skim Milk and Sucrose on The Viability of Lactic Acid Bacteria and Quality of Probiotic Cocoghurt Produced Using Starters. *Pakistan Journal of Biotechnology*; 16(1): 13-20.
Pato U, Yusuf Y, Panggabean IP, Handayani NP, dan Kusuma A, 2021. Viability of Lactic Acid Bacteria, Fatty Acid Profile and Quality of Cocoghurt Made Using Local and Commercial Starters During Fermentation. *International Journal of Agricultural Technology*; 17(3): 1001-1014.
Pereira ALF dan Rodrigues S, 2018. Turning Fruit Juice into Probiotic Beverages. In *Fruit Juices*; 279-287. Academic Press.
Peters OO, Afolabi MO, dan Makinde FM, 2023. Chemical, Physicochemical, and Sensory Properties of Yoghurt and Yoghurt Simulates Produced from The Blends of Cow Milk and Coconut Milk. In *IOP Conference Series: Earth and Environmental Science*; 1219(1): 012020.
Purwantiningsih TI, Bria MAB, dan Kia KW, 2022. Kadar Protein Dan Lemak Yoghurt yang Terbuat dari Jenis dan Jumlah Kultur yang Berbeda. *Journal of Tropical Animal Science and Technology*; 4(1): 66-73.
Putri R, Mursiti S, dan Sumarni W, 2017. Aktivitas Antibakteri Kombinasi Temu Putih dan Temulawak terhadap *Streptococcus mutans*. *Indonesian Journal of Mathematics and Natural Sciences*; 40(1): 43-47.
Qadi WS, Mediani A, Benchoula K, Wong EH, Misnan NM, dan Sani NA, 2023. Characterization of Physicochemical, Biological, and Chemical Changes Associated with Coconut Milk Fermentation and Correlation Revealed by 1H NMR-Based Metabolomics. *Foods*; 12(10): 1971.
Qiu W, Zhou Y, Li Z, Huang T, Xiao Y, Cheng, L., Peng X, dan Ren B, 2020. Application of Antibiotics/Antimicrobial Agents on Dental Caries. *BioMed Research International*; 2020: 1-11.
Raharjanti Z, Pramono YB, dan Al-Baarri AN, 2019. Nilai pH dan Kekentalan *Cocoghurt* dengan Penambahan Ekstrak Daun Stevia. *Jurnal Teknologi Pangan*; 3(2): 305-308.
Rahman FA, Haniastuti T, dan Utami TW, 2017. Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Etanol daun Sirsak (*Annona muricata* L.) pada *Streptococcus mutans* ATCC 35668. *Majalah Kedokteran Gigi Indonesia* 3(1): 1-7.
Rahman IR, Nurkhasanah K, dan Kumalasari I, 2019. Optimasi Komposisi *Lactobacillus bulgaricus* dan *Streptococcus thermophilus* pada Yogurt Terfortifikasi Buah Lakum (*Cayratia trifolia* (l.) domin) sebagai Antibakteri terhadap *Escherichia coli*. *Pharmaceutical Sciences and Research*; 6(2): 99-106.
Rahmayani N, Nazaruddin N, dan Amaro M, 2021. Pengaruh Konsentrasi Propolis terhadap Mutu Kimia, Mikrobiologi dan Organoleptik Yoghurt. *Jurnal Teknologi Pangan*; 15(1): 105-120.

Rasbawati R, Irmayani ID, Novieta N, dan Nurmiati N, 2019. Karakteristik Organoleptik dan Nilai pH Yoghurt dengan Penambahan Sari Buah Mengkudu (*Morinda citrifolia* L). *Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan*; 7(1): 41-43.
Sansawal R, Ahlawat U, dan Dhanker R, 2017. Yoghurt: A predigested Food for Lactose-Intolerant People. *International Journal of Current Microbiology and Applied Sciences*; 6(12): 1408-1418.
Sarah S, Indarti E, dan Yunita D, (2021). Pengujian Starter Yoghurt (*Streptococcus thermophilus* dan *Lactobacillus bulgaricus*) serta Ekstrak Jamblang (*Syzygium cumini* L.) pada Kasus Niyoghurt Jamblang yang Telah Rusak. *Jurnal Ilmiah Mahasiswa Pertanian*; 6(1): 37-43.
Sari P, Mentari INF, dan Kurniawan E, 2019. Aktivitas Antibakteri Etanol Spons Laut (*Calyspongia* sp) terhadap *Streptococcus mutans* Penyebab Karies Gigi. *Media of Medical Laboratory Science*; 3(2): 52-62.
Sarifuddin N, 2022. Formulasi dan Uji Aktivitas Antibakteri *Mouthwash* Ekstrak Daun Beluntas (*Pluchea Indica* Less) terhadap *Streptococcus mutans*. *Biolearning Journal*; 9(1): 9-12.
Sherlahwaty D dan Syarmalina NS, 2015. Analisis Kandungan Lemak dan Protein terhadap Kualitas *Soyghurt* dengan Penambahan Susu Skim. *Berkala Ilmiah Kimia Farmasi*; 4(2): 35-42.
Sieuwerts S, 2016. Microbial Interactions in The Yoghurt Consortium: Current Status and Product Implications. *SOJ Microbiology & Infectious Diseases*; 4(2): 1-5.
Suanda IW, 2018. Gerakan Masyarakat Hidup Sehat dalam Mencegah Terjadinya Penyakit Gigi dan Mulut. *Jurnal Kesehatan Gigi* (*Dental Health Journal*); 6(1): 29-34.
Sukmiwati M, Diharmi A, Mora E, dan Susanti E, 2018. Aktivitas Antimikroba Teripang Kasur (*Stichopus vastus* Sluiter) dari Perairan Natuna Kepulauan Riau. *Jurnal Pengolahan Hasil Perikanan Indonesia*; 21(2): 328-335.
Suwito MB, Wahyunitisari MR, dan Umijati S, 2017. Efektivitas Ekstrak Seledri (*Apium graveolens* L. var. secalinum Alef.) terhadap Pertumbuhan Bakteri *Streptococcus mutans* sebagai Alternatif Obat Kumur. *Jurnal kedokteran syiah kuala*; 17(3): 159-163.
Tandra TA, Khairunissa S, Sim M, dan Florenly F, 2020. Efek Penambahan Nanokitosan 1% Kedalam Berbagai Konsentrasi Ekstrak Kulit Kelengkeng *Streptococcus mutans*. *Jurnal Ilmiah Kesehatan Sandi Husada*; 11(1): 403–412.
Thompson W, Williams D, Pulcini C, Sanderson S, Calfon P, dan Verma M, 2021. Tackling Antibiotic Resistance: Why Dentistry Matters. *International Dental Journal*; 71: 450-453.
Tian H, Li B, Evivie SE, dan Sarker SK, 2018. Technological and Genomic Analysis of Roles of the Cell-Envelope Protease PrtS in Yoghurt Starter Development. *International Journal of Molecular Science*; 1(1): 1–17.
Wirawati CU dan Nirmagustina DE, 2022. Suplementasi Hidrolisat Glukomanan Tepung Porang (*Amorphophallus oncophillus*) pada Produk Minuman Sinbiotik. *Jurnal Penelitian Pertanian Terapan*; 22(1): 37-44.
World Health Organization, 2017. *Sugars and Dental Caries.* [Online] https://www.who.int/news-room/fact-sheets/detail/sugars-and-dental-caries. Diakses pada 30 Oktober 2022.
Yunita D, Varizki E, Rohaya S, Irfan I, dan Muzaifa M, 2023. Application of Optimized *Streptococcus thermophilus* and *Lactobacillus bulgaricus* on Coconut Milk in the Production of Niyoghurt. *Jurnal Natural*; 23(2): 131-138.
Yusriyani Y, Asfi D, dan Yuliastuti R, 2023. Uji Daya Hambat Ekstrak Etanol Daun Miana Merah (*Coleus benth*) terhadap *Staphylococcus aureus*. *Jurnal Kesehatan Yamasi Makassar*; 7(1): 10-16.
Zhi CK, Lani MN, Hamzah Y, Ahmad FT, dan Ubaidillah NHN, 2021. Characterisation of Lactic Acid Bacteria Isolated from Kefir Milk Made from Dairy and Non-Dairy Sources and Their Sensory Acceptance. *University Malaysia Terengganu Journal of Undergraduate Research*; 3(2): 37-50.
Zuhri R, 2019. Pengaruh Jenis Starter Bakteri Asam Laktat terhadap Produksi Asam Laktat dan Kadar Protein dalam Pembuatan Yoghurt Kacang Hijau (*Phaseolus radiatus* L.). *Eduscience Development Journal*; 1(2): 171-179.

Article History:
Received: 29 May 2024
Revised: 16 July 2024
Available online: 29 July 2024
Published: 30 September 2024

Authors:
Ananda Amalia Isnaini, Biology Study Program, Faculty of Mathematic and Natural Sciences, Universitas Negeri Surabaya, Jalan Ketintang Gedung C14 Surabaya 60231, Indonesia, e-mail: anandaamalia.20029@mhs.unesa.ac.id
Guntur Trimulyono,Biology Study Program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jalan Ketintang Gedung C14 Surabaya 60231, Indonesia, e-mail: gunturtrimulyono@unesa.ac.id

How to cite this article:
Isnaini AA, Trimulyono G, 2024. Antibacterial Activity of *Coconut Yoghurt* against *Streptococcus mutans* in Vitro. LenteraBio; 13(3): 393-401.