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Selection and Characteristics of Antimicrobial Starter Mix of Non-Probiotic and Probiotic in 'Kecipir' Yogurt

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Abstract

Background. Now, the role of functional food has become more important in maintaining and controlling public health. Yogurt can be an alternative, as it is familiar, well-liked, and has been reported by many for its functional properties.

Aims. The development of 'kecipir'ed 'kecipir's (*Psophacarpus tetragonolobus* L.) into food products is still relatively rare, even though it has great potential because it contains high nutrients and several bioactive components. The manufacture of 'kecipir's into yogurt, especially using lactic acid (BAL) bacterial starters, is a mixture of nonprobiotic yogurt (SY) starter and probiotic starter (SP), which is expected to improve its functional properties. One of the essential functional properties of yogurt is its antimicrobial properties.

Methods. This research was made in two stages. Phase I aimed to identify the best antimicrobial properties of the 7 BALs (2 SY and 5 BAL SP) against E. coli and Staphylococcus aureus. The antimicrobial activity test employed the agar diffusion method, which is measured by the diameter of the clear zone of inhibition around the sound hole in the agar medium. Phase II aims to determine the optimal combination of a 4% starter mixture (SY+SP) that produces the best antimicrobial and organoleptic properties in cheesecake yogurt.

Result. Phase I research produced four best BAL starters, namely two SY (BAL 1 *Lactobacillus bulgaricus* FNCC 0041 UGM and BAL 2 *Streptococcus thermophillus* ITB), as well as two SP (BAL 5 *Lactobacillus acidophillus* UNPAD and BAL 7 *Bifidobacterium* spp UNPAD). Phase II research obtained results that the antimicrobial properties were significantly different at the level of 5%, from the highest, namely SY4–SP0 starters at level a, SY1–SP3 starters at levels a, b, and combinations of SY3–SP1, SY2–SP2, and SY0–SP4 at level b. Organoleptically (hedonic), the combination of SY1–SP3 was the highest preferred by the panelists.

Conclusion. So the best mixed starter in this study is SY1–SP3, which comprises 1% of the SY starter mixture and 3% of the SP starter mixture.

Keywords: Functional Food, Yogurt, Breastfeeding, BAL, Probiotics, *E.coli*, *Staphylococcus aureus*, Antimicrobial.

INTRODUCTION

The need for functional food aligns with the trend of individuals seeking healthier conditions through natural means, such as consuming functional foods. Even Essa *et al.* (2023) noted that functional food today has a vital role in maintaining healthy living and reducing various risks from various diseases. Furthermore, Fekete *et al.* (2025) conducted a review of the role of functional food and its implications for public health.

Functional food is food that contains one or more functional components (bioactive) that have specific physiological functions, are not harmful, and beneficial to health (BPOM 2005). International bodies such as EFSA, FDA, and WHO explain that functional foods are those that contain bioactive components that contribute to the prevention and control of chronic non-communicable diseases, including cardiovascular disease, type two diabetes, and cancer (Fekete *et al.,* 2025).

Jatraningrum (2012) reported that trade in functional food products, nutraceuticals, and *dietary fiber* in the Americas reached USD 20.5 billion in 2004, estimated to be USD 100 billion in 2009. Soedarto (2008) reported that nutritionists estimate that probiotics and prebiotics are the fifth food trend of the Top 10 Food Trends *for 2008*. The production and marketing of prebiotic, probiotic, and synbiotic food products such as yogurt, fermented milk, baby food, and milk is increasing rapidly.

Yogurt is a functional food that is already known to the Indonesian people; it is expected to be one of the alternatives to meet the community's needs for functional food. There have been many reported functional properties of yogurt, such as: soygurt high in dietary fiber (Wening *et al.*, 2022), antioxidant properties of yogurt (Papadaki and Roussis, 2022), antioxidant and antimicrobial properties of yogurt (Auli *et al*., 2025), antimicrobial properties, physical and chemical characteristics of yogurt (Salama *et al*., 2019), and especially antimicrobial properties of yogurt (Pradana *et al*., 2023, Muraklina *et al*., 2022, Isnaini and Trimulyono, 2024, Vuran *et al*., 2021).

The antimicrobial properties of yogurt are important in balancing the gut microflora to maintain health. Adolfsson *et al.* (2004) found that the health benefits of yogurt, namely by balancing the intestinal microflora, help with lactose intolerance, help the body's immune system in the intestines, control colon cancer, and neutralize allergies in digestion. Rahayu *et al.* (2013) reported that symbiotic yogurt showed antidiarrheal activity against *enteropathogenic E. coli* as well as antioxidant activity.

‘kecipir’ed ‘kecipir’s (*Psophocarpus tetragonolobus* L.) are a *legume* that has been known for a long time in Indonesia (Budijanto *et al.*, 2011) and is recognized to be native to tropical regions (Nusifera *et al.*, 2011). ‘kecipir’s have great potential to be developed as functional foods & strategic foods, such as their high nutritional content and the presence of bioactive components.

The use of ‘kecipir’s in Indonesia is still minimal, such as as intermediate crops, vegetables (young leaves & young pods), and food (old seeds). BPS has not monitored ‘kecipir’s like other food crops such as corn, soybeans, peanuts, green beans, cassava or sweet potatoes. In fact, according to Rukmana (2000), Malaysia, the Philippines, Myanmar, and Nigeria have made ‘kecipir’s as a national food. Misra *et al.* (1987), as cited in Ningombam et al. (2012), also reported that in several developing countries, ‘kecipir’s have been used to overcome protein and calorie malnutrition.

The high protein and fat content of the partridge, especially in the seeds (Mohanty *et al*., 2013), can reach 50.7% dry base in fully ripe seeds (Ningombam et al., 2012), which is even higher than in some other foods. According to Rukmana (2000), ‘kecipir’s contain approximately 32.8 grams of protein and 27.0 grams of beef per 100 grams. Compared to soybeans, mung beans, and peanuts, ‘kecipir’s contain some better amino acids (Nusifera *et al.*, 2011).

Bioactive components of ‘kecipir’s include isoflavones, dietary fiber, and unsaturated fatty acids. There have been many studies that report the health benefits of isoflavones. According to Wahyuni (2010), raw ‘kecipir’s have a higher isoflavone content than yellow soybeans and chickpeas, by 0.21%, 0.18% and 0.15%, respectively. Homma *et al* (1983) reported that ‘kecipir’ed fat can reach 15.5%, of which 60.8% are unsaturated fatty acids, namely oleic (omega-6) 36.0% and linoleic (omega-9) 24.8%.

One of the developments of ‘kecipir’s is to make cheesecake yogurt. The presence of bioactive components in cheesecake is expected to enhance the functional properties of cheesecake yogurt compared to dairy product yogurt, including the presence of isoflavones, dietary fiber, and unsaturated fatty acids.

The commonly used yogurt starter culture is a blend of *S. thermophilus* & *L. bulgaricus*. However, based on several reports, such as Tamime & Robinson (2000) and Molin (2001), it is stated that most of these strains cannot survive well in the digestive tract (they are non-probiotic). So a probiotic starter was developed, namely a BAL starter that can survive the intestinal digestion.

Probiotics themselves provide health benefits by creating a balance of gut microflora and controlling pathogenic microbes (Chetana *et al.*, 2013). The addition of probiotics can also improve the immune system, reduce lactose intolerance cases, control diarrhea, and lower cholesterol levels (Scheinbach, 1999; Hussain *et al.*, 2009). In terms of organoleptics (taste and aroma), probiotic yogurt is better than non-probiotic or natural yogurt (Hussain *et al.*, 2009), and the addition of probiotics to non-probiotic starters can improve the taste and aroma of yogurt (Nizori *et al.*, 2007). *Bifidobacterium* and *Lactobacillus* are probiotics that are often studied and applied to probiotic foods.

Prebiotics are carbohydrates that cannot be digested but can be fermented by bifidobacteria and lactobacilli, which can increase intestinal bacterial activity (Gibson and Foberfroid, 1995; Ziemar & Gibson, 1998, as cited in Miremadi, 2012). Bacteria that can digest prebiotics are probiotics. Prebiotics have a positive impact on probiotics and health for their hosts.

Old ‘kecipir’ed seeds can contain as much fiber as 3.7 – 16.1 grams per 100 grams (Bostid, 1981). In addition to being needed in the digestive process, dietary fiber is also important as a prebiotic, which is a food for probiotics in the digestive tract.

Symbiotics are mixtures of probiotics and prebiotics that can provide additional benefits to their hosts, such as enhancing the resistance and growth of probiotic bacteria, selectively stimulating probiotic microbes, and inhibiting certain microbes, thereby improving the host's health (Gibson and Roberfroid, 1995, as cited in Miremadi & Shah, 2012). The ‘kecipir’ed yogurt produced is expected to be symbiotic so that it will improve its functional properties, including antimicrobial properties and starter durability during digestion and storage.

Another important functional property of yogurt is its ability to inhibit the growth of pathogenic and destructive bacteria. These inhibitions are due to the presence of antimicrobial substances produced by BAL, such as bacteriocins. According to Majeed *et al*. (2011) in Dobson (2012), bacteriocins can act as killer peptides by competing directly with other strains or pathogenic bacteria. The use of BAL starter bacteriosin-positive strains is expected to improve the functional properties of antimicrobial yogurt cheese.

Shagti (2012) has conducted a study on cockpit yogurt, which has inhibitory properties against Salmonella typhi. *Escherichia coli* is a bacterium that is often used as an indicator of sanitation or *food hygiene*, because the presence of *E.coli* is an early indication of the presence of other bacterial contamination in food and beverages (Puspitasari, 2013). *E. coli* can cause diarrhea, fever, abdominal cramps, and vomiting (Entjang, 2003, in Bambang et al., 2014), and can even cause synitis, which is inflammation of the mucous membranes of the bladder (Melliawati, 2009). For this reason, it is essential to investigate the antimicrobial properties of ‘kecipir’ed yogurt against E. coli.

In this case, a study is needed that focuses on the application of ‘kecipir’s as a food product, especially as a functional food, namely yogurt, which is familiar and has many proven health benefits, and is fermented with a mixture of yogurt starter (non-probiotic) with probiotic starters, so that its characteristics and functional properties are expected to be better.

METHODS

Phase I Research

Phase I research is aimed at looking at the antimicrobial properties of 7 (seven) BAL Starter Yoghurt (Non Probiotic), namely (1) *Lactobacillus bulgaricus* FNCC 0041 UGM, (2) *Streptococcus thermophillus* ITB, and Probiotic Starters, namely: (3) *Lactobacillus lactis* ITB, (4) *Lactobacillus acidophilus* ITB, (5) *Lactobacillus acidophilus* UNPAD, (6) *Lactobacillus plantarum* UNPAD, (7) *Bifidobacterium* spp UNPAD. The test bacteria used are *E.coli* and *Staphylococcus aureus.*

BAL stock is stored by being scratched on MRSA oblique agar (De Man, Rogosa, Sharpe Agar) at freezing temperatures (-18 °C). The activation of the BAL starter is carried out by first being scratched on MRSA so that it is tilted (24 hours, temperature 35 °C), then grown on MRSB (De Man, Rogosa, Sharpe Broth), for 24 hours, temperature 35 °C. The bacteria tested, stored on NA (Nutrient Agar) are skewed at freezing temperatures. Activation of the test bacteria was first grown on the NA to be oblique (24 hours, temperature 35 °C), and continued in the BHI Broth (24 hours, temperature 35 °C). The antibacterial properties are carried out by the agar diffusion method, where the activated test bacteria are then inoculated as much as 0.2% into a liquid PCA (Plate Count Agar), then distributed about 15 ml into a sterile petri dish. After the PCA freezes, a hole is made about 0.5 cm. A total of 50 µl of BAL filtrate that has been grown and filtered through 0.22 µm sterile millipore paper is inserted into the hole that has been made, incubated (24 hours, temperature 35 °C), and the zone of inhibition (in cm) is calculated.

Phase II Research

The Phase II study is aimed at looking at the composition of the best starter mixture, Starter Yoghurt (SY) with the best Probiotic Starter (SP). SY and SP each consist of two BALs with a 1:1 composition. The total SY+SP is 4%, so that the combination of the mixed starter: SY0+SP4 (SY 0% and SP 4%), SY1+SP3, SY2+SP2, SY3+SP1, and SY4+SP0. The observed response consisted of antimicrobial properties of cheesecake yogurt, organoleptic, BAL amount, and pH value. The antimicrobial properties of ‘kecipir’ed yogurt were tested by separating the BAL using a centrifuge and then filtering it through 0.22 μm sterile Millipore paper.

‘kecipir’ed Milk Making

A total of 333 grams of cleats were soaked with the addition of 0.15% NaHCO3 for 12 hours, then steamed at a temperature of 80-90 °C for 5 minutes and peeled. The skinless seeds are crushed and extracted using a soy milk juicer using hot water (at least 55 °C) as much as 500 ml, and the filtrate (solution a) is taken. Other auxiliary ingredients are CMC 0.05%, gelatin 0.2%, skim milk 5%, sucrose 5% and lactose 2% dissolved (solution b). Next, solutions a and b are combined to a volume of 1000 ml and mixed with a blender (into milkweed milk). A total of 200 ml of ‘kecipir’ed milk is put into a 500 ml sterile bottle, then pasteurized with an autoclave at a temperature of 105 °C for 5 minutes. The bottle is immediately cooled in the laminar flow, after the temperature is less than 45 °C, ready to inoculate the SY-SP mixed BAL starter.

DISCUSSION

Phase I Research

The BAL activated in MRSB can be seen in Figure 1a. The growth of BAL is seen from the murky media. In MRSB media, the control (without BAL inoculation) was clear in color, depicting the absence of growth. The antimicrobial activity test was performed by separating BAL cells by filtration using 0.22 μm Milipore paper (Figure 1b).

(a) (b)

Figure 1. probiotic and non-probiotic BAL tested (a) BAL after grown in MRSB and (b) BAL free filtrate (BAL) grown in MRSB filtered paper millipore 0.22 μm).

(a) From left to right: BAL 1, BAL 2 ... BAL 7, MRSB control
(b) From left to right: BAL 7, BAL 6 ... BAL 1

The test pathogen bacteria, from the NA tilted agar medium, are first grown in BHI (Brain Heart Infusion) Broth, then inoculated into liquid PCA and immediately poured into a petri dish. After freezing, a hole (well) is made and the well is ready to be inoculated with BAL filtrate or BAL cell-free filtrate, which can be seen in Figure 2.

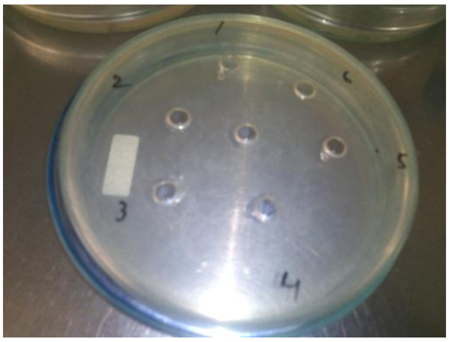
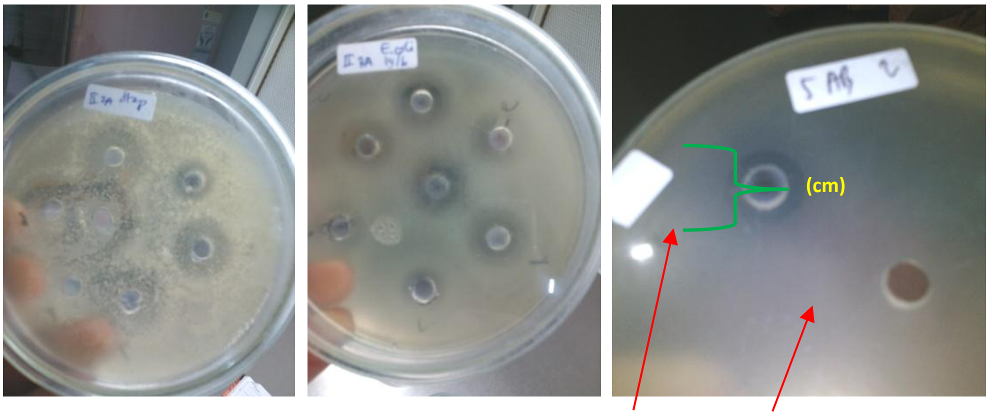


Figure 2. Agar Media (PCA) that has been inoculated with bacteria *Pathogen* (*E.coli* or *Staphylococcus aureus*), before BAL free filtrate is added and has not been incubated.

After the hole (well) in the PCA is inserted, 50 μl of BAL-free filtrate is added, and then the medium is incubated at 35 °C. Pathogenic bacteria will grow in agar medium, but the addition of BAL filtrate will inhibit their growth around the hole, as indicated by the absence of pathogenic bacteria gro'kecipir' around the hole or appearing as a clear zone. The antimicrobial properties of BAL were determined by calculating the width of the apparent inhibition zone as a clear area without the growth of the test bacteria, and were calculated in cm. The BAL inhibition zone is visible in Figure 3. The test control uses MRSB media, sho'kecipir' no inhibition zone, as illustrated in Figure 3d.



(a) (b) (c) (d)

Figure 3. Clear zone is the zone of inhibition by BAL: (a) against *E.coli*, (b) against *Staphylococcus aureus*, (c) diameter zone Inhibition and (d) Control (MRS Broth)

BAL's antimicrobial properties against *E.coli* can be seen in Figure 4, from the largest are: BAL 7 (1.41 cm), BAL 6 (1.33 cm), BAL 5 (1.30 cm), BAL 1 (1.26 cm), BAL 4 (1.22 cm), BAL 3 (1.19 cm), and the lowest BAL 2 (1.13 cm). Nevertheless, based on the analysis, it is not significant. As shown in Figure 5, the largest are: BAL 5 (2.15 cm), BAL 7 (2.07 cm), BAL 6 (2.06 cm), BAL 1 (1.97 cm), BAL 4 (1.87 cm), BAL 3 (1.78 cm), and the lowest BAL 2 (1.44 cm).

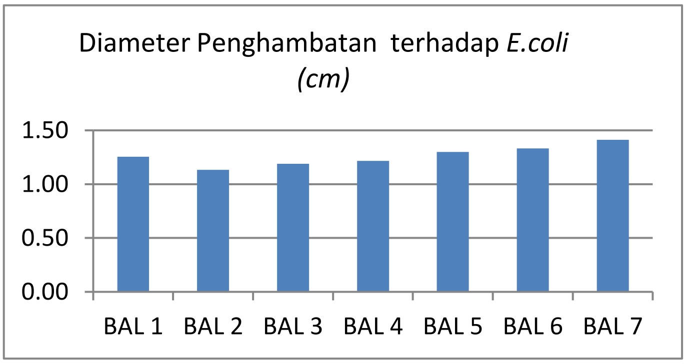


Figure 4. Graph of the Antimicrobial Properties of BAL against *E.coli*

Information: BALL 1: *Lactobacillus bulgaricus* FNCC 0041 UGM, BAL 2: *Streptococcus thermophillus* ITB, BAL 3: Lactobacillus lactis ITB, BAL 4: Lactobacillus acidophilus ITB, BAL 5: Lactobacillus acidophilus UNPAD, BAL 6: Lactobacillus plantarum UNPAD, BAL 7: *Bifidobacterium* spp. UNPAD

Based on anava tests, BAL's antimicrobial properties against *S. aureus* is a real difference in 1%. Based on the Duncan test, the real difference is obtained for the seven BALs, from the highest namely: BAL 5, BAL 7, BAL 6, BAL 1, BAL 4 (level a), BAL 3 (level a, b) and BAL 2 (level c).

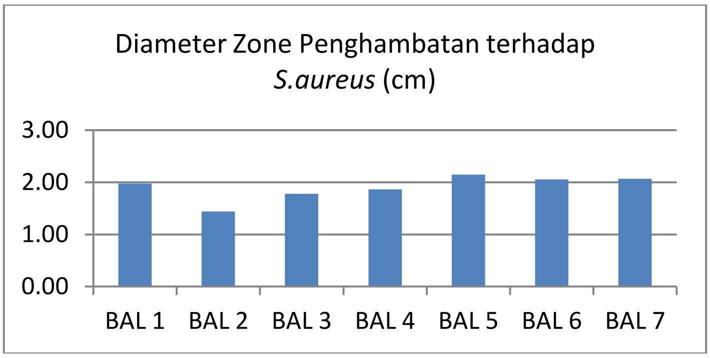


Figure 5. Graph of Antimicrobial Properties of BAL against *Staphylococcus aureus*

Information: BALL 1: *Lactobacillus bulgaricus* FNCC 0041 UGM, BAL 2: *Streptococcus thermophillus* ITB, BAL 3: *Lactobacillus lactis* ITB, BAL 4: *Lactobacillus acidophilus ITB, BAL 5 : Lactobacillus acidophilus* UNPAD, BAL 6: *Lactobacillus plantarum* UNPAD, BAL 7: *Bifidobacterium* spp. UNPAD

The presence of an inhibition zone illustrates the antimicrobial properties of BAL. The antimicrobial properties of BAL are attributed to the production of lactic acid, other organic acids, H2O2, and bacteriocins. Mayo *et.al*. (2010) and Garcia-Cano *et.al*. (2014) in Nurhayati *et.al* (2020), reported that antibacterial compounds during BAL fermentation can be in the form of organic acids (lactic acid and acetic acid), diacetyl, ethanol, hydrogen peroxide, reuterin, acetaldehyde, acetone, carbon dioxide, and bacteriocin.

From the results of the study, it can be seen that the antimicrobial properties of all BAL starters are higher against *S. aureus* (gram-positive bacteria) than against *E.coli* (gram-negative bacteria). This is in line with research conducted by Nurhayati *et al*. (2020), which is because the E.coli cell wall is gram-negative and has a more complex cell wall structure.

Based on the best antimicrobial properties against the two pathogenic bacteria, the selected BAL is: BAL 1 (*Lactobacillus bulgaricus* FNCC 0041 UGM) and BAL 2 (*Streptococcus thermophillus* ITB) as **SY,** as well as BAL 5 (*Lactobacillus acidophilus* UNPAD) and BAL 7 (*Bifidobacterium* spp. UNPAD) as **SP.**

Phase II Research

The Phase II study aimed to see the characteristics of the best 'kecipir'ed yogurt, using a SY mixed starter (BAL 1 and BAL 2 ratio 1:1) with a SY mixed starter (BAL 5 and BAL 7 ratio 1:1). The total starter of SY and SP is 4%, with a combination of 5 (five) combinations, namely: SY4-SP0, SY3-SP1, SY2-SP2, SY1-SP3 and SY0-SP4. Its fifth antimicrobial property against E. coli can be seen in Figure 6, and the inhibitor diameters are 0.91 cm, 0.81 cm, 0.81

cm, 0.86 cm, and 0.75 cm, respectively. Based on the Anava tests, it has antimicrobial properties. The real difference is at the level of 5%, and based on the Duncan test, the real difference is obtained, namely: **a** is a mixture of SY4–SP0, **a,b** is SY1–SP3, and b is: SY3–SP1, SY2–SP2, and SY0–SP4.

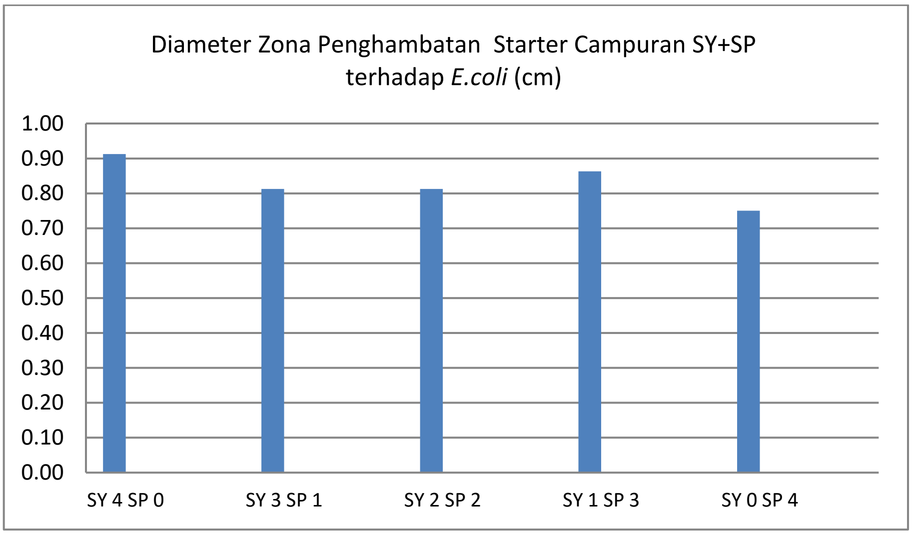


Figure 6. Antimicrobial Properties Graph of Mixed Starter Mixes between Yogurt Starter (SY) with Probiotic Starter (SP) against *E.coli*

Information: SY consists of BAL 1 (*Lactobacillus bulgaricus* FNCC 0041 UGM) and BAL 2 (*Streptococcus thermophillus* ITB). SY consists of BAL 5: Lactobacillus acidophilus UNPAD and BAL 7: *Bifidobacterium* spp. UNPAD

Based on the antimicrobial properties of the five mixed starter combinations above, the best starter combination is **SY1–SP3** (1% starter yogurt, consisting of *Lactobacillus bulgaricus* FNCC 0041 UGM and *Streptococcus thermophilus* ITB, and 3% probiotic starter, consisting of *Lactobacillus acidophilus* UNPAD and *Bifidobacterium spp.).* UNPAD). Although SY4 exhibits the highest antimicrobial properties–SP0, they are not utilized because they do not contain SP (probiotic starters). The presence of probiotic starters in yogurt is expected to improve other functional properties of the yogurt products produced.

The growth of SY and SP mixed starter in 'kecipir'ed milk that produces 'kecipir'ed yogurt can be seen in Figure 7. Observations were made at the 0, 6, 12, 20, and 23rd hours. The total number of BAL starters from the various starter combinations on **0th hour is about 106 CFU per gram** (6.38, 6.36, 6.44, 6.15 and 6.43 respectively log10.cfu.ml^{-1} for SY4-SP0, SY3-SP1, SY2-SP2, SY1-SP3 and SY0-SP4), as well as on **The 23rd hour is about 1010 CFU per gram** (respectively 10.80, 11.90, 10.38, 10.00 and 10.50 for SY4-SP0, SY3-SP1, SY2-SP2, SY1-SP3 and SY0-SP4). The amount of BAL at the end of fermentation from all starter combinations meets the yogurt standard, which is at least 107 cfu per ml (SNI 2981: 2009).

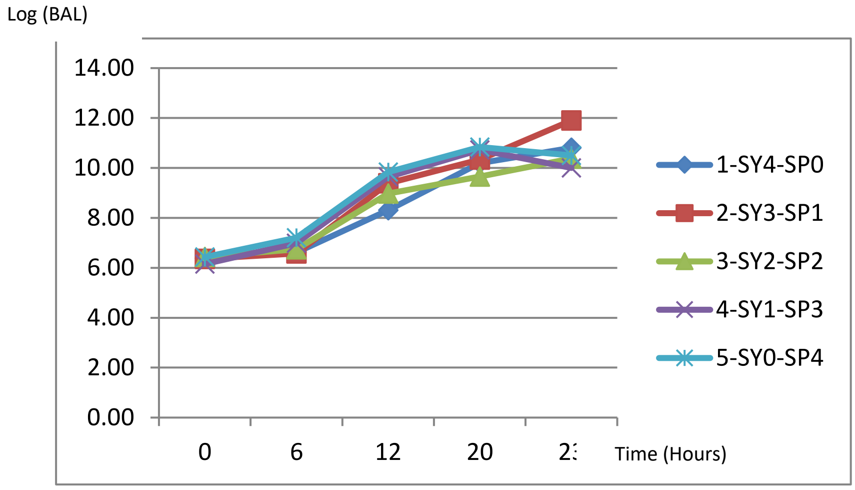


Figure 7. BAL Growth Chart on 'kecipir'ed Yogurt with Mixed Starter Combination between SY and SP

From Figure 7 above, the overall growth pattern of BAL for all starter combinations, there is a trend that the adaptation phase occurs until the 0th to 6th hour, the exponential phase between the 6th to the 20th hour, and from the 20th hour onwards to the stationary phase.

The important thing about probiotic sifap is its viability during storage, as Savari's research reports *et al*. (2014), namely making yogurt from milk powder that is reconstituted so that it contains 12.0% non-fat milk solids (MSNF – *Milk Solid Non-Fat*), using mixed cultures, namely **SY** (*Streptococcus thermophilus, Lactobacillus delbrueckii* Spp. *bulgaricus*) and **SP** (*Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12). Starter inoculations were 6.84, 7.16, 6.48, and 6.3 log10cfu.ml-1 to S. thermophilus and *L. delbrueckii spp. bulgaricus, L.acidophilus and B.lactis BB-12*). The test was carried out for up to 21 days of storage at 4 °C, and the number of final microbes was 8.70, 6.30, 6.93, and 6.79, respectively, log10cfu.ml^{-1}. 'kecipir'ed yoghurt in this study, if the same storage is carried out (21 days, temperature 4oC), then the number of BAL that is still alive is very likely to be close to or above the figure proposed by Savari *et al*. (2014), with considerations: a) similar starter composition, b) the nutritional composition of this study is more complex, c) the highest number of mixed microbes in this study can reach above 10 log10cfu.ml^{-1} (at the 20th & 23rd hour) – while Savari's research *et al*. (2014) maximum reaches 8.77 log10cfu—ml-1 (14th day). Further research is urgently needed to demonstrate the viability of starters at longer and more extreme storage, such as at freezing temperatures.

The pH value at the beginning of fermentation (milkshake mixture) was 6.5-6.6 for all treatments, and there was almost no change at the 6th hour of fermentation (in the range of 5.5 – 6.5). A pH value of less than 4.5 for all new treatments was achieved at the 12th hour of fermentation, specifically a value of 4.2 (SY4-SP1 & SY3-SP1), 4.3 (SY0-SP4), and 4.5 (SY2-SP2 & SY3-SP1). At the end of fermentation, i.e., the 23rd hour, all treatments reach a pH of 4.0. Savari *et al*. (2014) reported the pH value of fermented yoghurt made from reconstituted milk powder, with the total content of non-fat milk solids (MSNF – Milk Solid Non-Fat) 12.0 %, i.e., it reaches a pH value of 4.5 at 4 hours of fermentation.

The pH value at the end of the fermentation of this experiment reached 4.0. According to Aswal *et all* (2012), the ideal pH value for finished yogurt products is 4.0-4.1, and after fermentation, the pH value of yogurt that is ready to be consumed must reach 4.0. According to him, the pH value of yogurt will increase along with the amount of skim milk added. Yogurt is made from a mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, with Streptococcus playing a role in lowering the pH to 5 sec, and *Lactobacillus* playing a role in further lowering the pH to pH 4.5.

Organoleptic (hedonic) test parameters include color, taste, aroma, and viscosity. In the organoleptic test, in addition to testing yogurt with five combinations of SY+SP, it was also compared with 'kecipir'ed milk (which was not fermented). For the overall organoleptic value from the average calculation of all parameters (color, taste, aroma and viscosity), the average value is 3.2, and from the highest to lowest organoleptic average value is: 1) First **SY1-SP3** (with a value of 3.4), 2) Second **SY0-SP0** ('kecipir'ed milk without starter & without fermentation, with a value of 3.33), 3) Third **SY2-SP2** (with a value of 3.29), 4) Fourth **SY3-SP1** (value 3.20), 5) Fifth **SY0-SP4** (all consist of probiotic cultures and without yogurt cultures, with a value of 3.14), 6) and finally are **SY4-SP0** (all yogurt cultures, without probiotic cultures, with a value of 3.11). The comparison of the average organoleptic hedonic values can be visually seen in Figure 8. The composition of the starter selected from this stage of the study is a combination of **SY1-SP3** (a mixture of 1% yogurt starter and 3% probiotic starter %), which is the highest average organoleptic value, although the Anava test was not significant.

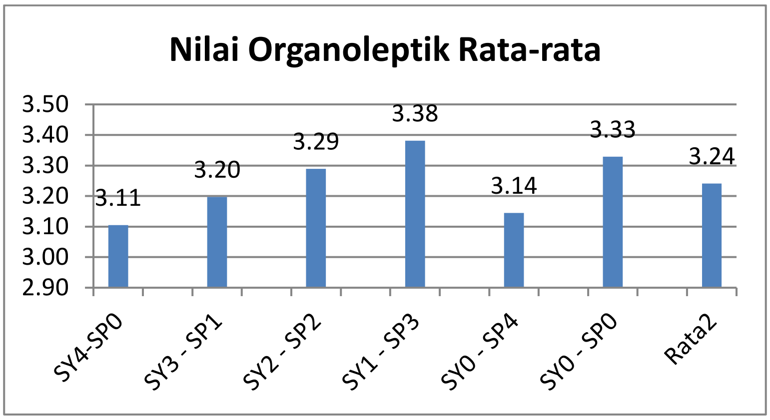


Figure 8. Average Organoleptic Values (color, taste, aroma, viscosity) of 'kecipir'ed yogurt with SY mixed starter (yogurt starter) and SP (probiotic starter). *Note: SY0-SP0 = control (milkweed milk)*

From these results, it can also be seen that mixed cultures consist of only **SY** (*Lactobacillus bulgaricus* FNCC 0041 UGM and *Streptococcus thermophillus* ITB), or mixed cultures consisting only of **SP** (*Lactobacillus acidophillus* UNPAD and *Bifidobacterium* spp. UNPAD), namely SY4-SP0 and SY0-SP4, both of which resulted in lower mean organoleptic hedonic values compared to the starter of the mixture **SY** and **SP** (i.e., SY1-SP3, SY2-SP2, SY3-SP1). In line with Nizori's report *et.al.* (2007), the addition of probiotic cultures (L.acidophilus) against yogurt culture (SY), namely *L. Bulgaricus* and *S.thermophillus*, can increase the organoleptic value of the resulting soygurt.

Ersan and Kurdal (2014) also reported that the manufacture of bio-yogurt uses mixed cultures between SY yogurt starters (*Streptococcus thermophillus* and *Lactobacillus bulgaricus*) with SP probiotic starter (*L.acidophillus*, *Bifidobacterium* Spp, *L.lactis* and *L.casei*) can increase organoleptic value. The composition of the SY+SP starter is: **a)**. *S.thermophillus* + *L. Bulgaricus*, **b)**. *S.thermophillus* + *L.acidophillus* + *Bifidobacterium* Spp. **c)** *S.thermophillus* + *L.acidophillus* + *L.lactis* + *Bifidobacterium* Spp. **d)** *L.acidophillus* + *Bifidobacterium* SPP. and **e).** *L.acidophillus* + *L.lactis* + *L.casei*. The highest sensory value result is a combination **c,** and the lowest is the starter combination **e**. According to Ersan and Kurdal (2014), the sensory or organoleptic value of yogurt is influenced by: microbiological factors, process parameters in yogurt making, source or type of milk, and the presence of additives used.

In this Phase II study, the parameters observed include antimicrobial properties, BAL growth, pH values during fermentation and organoleptic properties (color, taste, aroma and viscosity), so the selected starter combination is **SY1-SP3**, namely SY (yogurt starter) as much as 1% and SP (probiotic starter) as much as 3% of the volume of milk formulation milk. **SY** consists of BAL 1 (*Lactobacillus bulgaricus* FNCC 0041 UGM) and BAL 2 (*Streptococcus thermophillus* ITB), with a ratio of 1:1. And **SP** consists of BAL 5 (*Lactobacillus acidophilus UNPAD) and BAL 7 (Bifidobacterium spp. UNPAD) with a ratio of 1* 1.

In line with the report of Shilpi & Kumar (2013), they make yogurt from milk (*toned milk*), which is added soy milk and mango pulp and inoculated by **Starter Yogurt** (ST = *S. thermophilus* NCDC 074 and LB = *L. delbrueckii spp bulgaricus* NCDC 009) and **Probiotic Starter** (BB = *Bifidobacterium bifidus* NCDC 255 & LA = *Lactobacillus acidophilus* NCDC 015). From the observation of physicochemical and sensory characteristics, the optimal formulation of the starter composition for ST:LB:BB:LA was determined to be 1.75%: 1.95%: 2.44%: 1.37%.

CONCLUSIONS

Tested antimicrobial properties of two BAL Starter Yoghurt **(SY)** Nonprobiotics and five BAL Probiotic Starters. BAL Nonprobiotic Yogurt Starter is **BAL 1**: *Lactobacillus bulgaricus* FNCC 0041 UGM and **BAL 2**: *Streptococcus thermophillus* ITB. BAL Probiotic Starter **(SP): BAL 3: Lactobacillus lactis ITB, BAL 4: Lactobacillus acidophilus ITB, BAL 5: Lactobacillus acidophilus UNPAD, BAL 6**: *Lactobacillus plantarum* UNPAD, and **BAL 7**: *Bifidobacterium* spp. UNPAD. All seven have antimicrobial properties against bacteria gram-negative bacteria, namely E. coli (although not significantly different), and against gram-positive bacteria, namely *Staphylococcus aureus* (the difference is very real). The best BAL as a mixed starter for making ‘kecipir’ed yogurt is: SY starter (BAL 1 and BAL 2 = 1:1) and SP (BAL 5 and BAL 7 = 1:1).

Furthermore, ‘kecipir’ed yogurt has been made using 4% mixed starter from the best BAL above, with a combination of SY4%-SP0%, SY2%-SP2%, SY1%-SP3% and SY0%-SP4%. The best antimicrobial properties against the test bacteria E. coli are observed in the SY1% - SP3% mixed starter. In line with the hedonic test, the ‘kecipir’ed yogurt that the panelists liked the most was yogurt from the SY1%-SP3% starter mixture, although it was not significant. At the beginning of fermentation, yogurt makes all quantities of BAL in all combinations above 6 log10 cfu/mL and above 10 log10 cfu/mL (meeting probiotic standards based on SNI 2981:2009).

It is important to conduct further research, namely its more specific functional properties related to health, such as the biocellular mechanisms that occur, in order to be more effective in its effects.

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