
DETECTION OF LACTIC ACID BACTERIA (LAB) IN SOAKING WATER OF ARABICA COFFEE CHERRIES ON MOUNT SINDORO

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ABSTRACT

Coffee is one of the major commodities in Indonesia's agricultural sector, where Indonesia is the fourth largest coffee producer in the world. Arabica coffee is the only type of coffee produced on Mount Sindoro. The used water is wasted in the sewers when washing and sorting coffee. Even though the skin covering the cherry flesh is likely to contain natural microorganism life since the coffee cherries grow during ripening on the tree. Therefore, this study aims to determine whether there is the presence or absence of Lactic Acid Bacteria (LAB) groups in the water used to soak the Arabica coffee cherries. The research method was carried out with 200 ml of water samples soaked in Arabica coffee cherries for 4 hours, isolating microorganisms from the 10⁻¹ to 10⁻³ dilution series of soaking water using the spread plate method and reinoculation to obtain pure LAB cultures, then detected macroscopically through observation of colony morphology and microscopically detected, including physiological and biochemical reaction tests. Data analysis was conducted in a fully descriptive-qualitative manner concerning Bergey's Manual of Determinative Bacteriology and other books supporting the detection of the BAL groups obtained. The study results obtained three isolates, but only one isolate was detected as LAB. The isolate belongs to the Streptococcaceae family's LAB group- the Lactococcus genus.

keywords: soaking water, lactic acid bacteria, arabica coffee cherries, detection.

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INTRODUCTION

Coffee is one of the major commodities in Indonesia's agricultural sector, where Indonesia is the fourth largest coffee producer in the world (with an average production range of 630-640 thousand tons/year). Meanwhile, in 2020, Indonesia produced 762,380 tons of coffee from a total land area of 1,250,452 Ha (Firmansyah et al., 2023). Especially in Central Java, total coffee production in 2020 was 26,179 tons, dominated by Community Plantations with 25,999 tons from 46,602 Ha of land area, the rest by Large State Plantations with 145 tons from 961 Ha of land area and by Plantations Large Private Sector is 35 tons from 194 Ha of land area. This data includes two types of coffee, which are large commodities, namely Arabica coffee (*Coffea arabica* Linn) and Robusta coffee (*Coffea canephora* Pierre).

One area is even a stratovolcano (semi-active/type B stratovolcano) located in Central Java, namely Mount Sindoro, which is in Pagergunung Village, Tlamp District, Temanggung Regency (northwest). Mount Sindoro has a height of 3,340 meters above sea level (Temanggung Regency Government, 2015). Therefore, Mount Sindoro is highly utilized in the agricultural and plantation sectors, including Arabica coffee. Arabica coffee is the only type of coffee produced on Mount

Sindoro. Arabica coffee has a slightly oval green bean morphology, contains 0.8-1.4% caffeine, 6-9% sugar, and 15-17% essential oil, and can grow optimally at 1000-2000 meters above sea level. Apart from coffee, Mount Sindoro is also planted with tobacco and vegetables (Temanggung, 2014). Based on data from the Temanggung Regency Central Statistics Agency, Kledung District produced 282.00 tonnes of Arabica coffee in 2018, which decreased in 2019 to a production figure of 188.16 tonnes (BPS, 2020). Kledung Sub-District is the closest sub-district to the slopes of Mount Sindoro. Most of its people work as farmers, making many places for coffee production, including Java Sindoro Processing owned by Mr. Arifin. Java Sindoro Processing alone can usually process an average of 50 tons/year of Arabica coffee production.

The processing of coffee on Mount Sindoro is generally done with the honey process, which is included in the wet processing process. The honey process begins with peeling the skin and flesh of the coffee cherries (commonly called cherries), leaving the coffee beans with their horn skin and mucilage (mucilage). This aims to ensure that during the drying/drying process, it can stimulate the start of the natural fermentation process in the coffee beans, which can produce organic acids to be absorbed by the coffee beans, thereby creating a typical fruity taste of fruit that is close to the taste produced by the natural process. or dry processing (Dalimunthe et al., 2021). The reactions that occur during the natural fermentation process include three important reactions as follows:

1. Breaking down the muscles so that the coffee bean horn skin can be easily removed from the coffee bean after drying.
2. The glycolysis reaction occurs.
3. There is a change in the color of the horn skin and epidermis for the better (Puspaningrum et al., 2018).

The mucilage contains 4.1% sugar, 84.2% water, 8.9% protein, 0.9% pectic acid, and 0.7% ash (Dalimun et al., 2021). Because of this, it supports the growth of microorganisms, especially lactic acid bacteria, and even putrefactive bacteria can also grow easily. Therefore, it requires good and correct handling and storage.

It should be noted that before stripping the skin and flesh of the cherries, the cherries are soaked as well as sorted using water, which water acts as an auxiliary medium in determining the quality of the cherries, namely cherries floating on the surface of the water are low-quality cherries/coffee cherries. While those sinking to the bottom of the water are high-quality coffee cherries/cherries. This is based on the weight and density of the cherries/coffee cherries themselves. After that, the water is wasted into the sewer. Even the skin covering the cherry flesh is most likely to contain the life of microorganisms that grow naturally since coffee cherries grow during ripening on the tree. When soaking is done, microorganisms can likely transfer from the cherry skin to the water so that the used soaking water contains natural microorganisms from the cherries, which can be used as isolates. Therefore, this can be innovated to create a liquid inoculum in the form of a lactic acid bacteria starter or with other microorganisms commonly used in food biotechnology as an auxiliary fermenter which is expected to also improve the taste produced by coffee beans in the future so that it can also improve quality and price at coffee products produced.

The Lactic Acid Bacteria (LAB) group is known as the best microorganism in the fermentation process in food because many and almost all fermented foods use this group. Apart from that, because LAB has met GRAS (Generally Recognized As Safe) status, it is safe for human consumption

and digestion, so that it can be applied as a probiotic agent (Putri & Kusdiyantini, 2018). This is because LAB is naturally found in many foodstuffs as well as in the gastrointestinal and urogenital tracts of humans and animals (Sujaya et al., 2016). The BAL group has a morphology in the form of cocci (round) or rod (bacillus) cells, arranged in pairs or chains, and are Gram-positive bacteria and do not have spores. Other properties include being non-motile, facultatively anaerobic, catalase-negative, and being able to produce lactic acid by fermenting carbohydrates. Therefore, LAB is always used in the fermentation process because it can produce acid, which can inhibit the growth of pathogenic microorganisms and food spoilage bacteria (Putri & Kusdiyantini, 2018), which is also supported by the production of hydrogen peroxide, diacetyl, or phenylacetic acid, and bacteriocin (Dewi, 2014).

The purpose of the study "Detection of Lactic Acid Bacteria (LAB) in Water Used for Soaking Arabica Coffee Cherries at Mount Sindoro" is to determine whether there is the presence of Lactic Acid Bacteria (LAB) and the Lactic Acid Bacteria (LAB) group in the water used for soaking cherries. Arabica coffee on Mount Sindoro.

METHOD

The research was conducted from September to November 2022. The research was conducted at the Microbiology Laboratory, Faculty of Biology, Satya Wacana Christian University, Salatiga. The tools used in this study included test tubes, test tube racks, micropipette and blue tips, round loop needles, straight loop needles, L rods, bunsen, vortex, petri dishes, analytical scales, incubators, autoclaves, Erlenmeyer flasks, microscopes, glass objects, Biological Safety Cabinet, pipettes, volume pipettes and pileus, refrigerator, plastic wrap, markers, aluminum foil, pH-meter, and hot plate-stirrer.

The materials used in this research include water used to soak Arabica coffee cherries (Arabica coffee picked directly from coffee trees in coffee plantations on Mount Sindoro), de Man's media, Rogosa and Sharpe Agar (MRSA), and de Man's media. , Rogosa and Sharpe Broth (MRSB), Nutrient Agar (NA) media, Tryptic Soy Agar (TSA) media, Mannitol Salt Agar (MSA) media, Simmon's Citrate Agar (SCA) media Triple Sugar Iron Agar (TSIA) media, Methyl media Red-Voges Proskauer (MR-VP), physiological NaCl 0.85%, sterile distilled water, spirit oil, immersion oil, crystal violet, iodine, alcohol 95%, safranin, malachite green 5%, H₂O₂ 3%, Methyl Red reagent, 40% KOH, and 5% α -naphthol solution. The work steps include sample preparation, equipment sterilization, and media creation.

Data analysis was carried out completely qualitative-descriptive. Data obtained from the Lactic Acid Bacteria (LAB) isolate test results included observations of colony morphology, cell shape, Gram staining test, spore staining test, motility test, catalase test, citrate test, Triple Sugar Iron (TSI) test, Methyl Red-Voges test Proskauer (MR-VP), and the mannitol test are presented in tabular form and accompanied by pictures which are then compared with Bergey's Manual of Determinative Bacteriology along with other books that support the detection of the BAL groups obtained.

RESULTS AND DISCUSSION

Isolation of Lactic Acid Bacteria (LAB) and Macroscopic Detection

Isolation of microorganisms is a technique that aims to move mixed colonies of microorganisms from their environment, as well as to separate colonies of microorganisms from one

another from their environment to artificial growth media so that colonies of similar microorganisms are obtained, which are called pure cultures. In a narrow sense, isolation is a technique for growing microorganisms outside their natural environment (Romadhon Badaring et al., 2020). The isolation carried out in this research was in the form of bacteria, especially Lactic Acid Bacteria (LAB) from the skin of Arabica coffee cherries (*Coffea arabica*) which were cultivated at an altitude of approximately 2000 meters above sea level on Mount Sindoro by soaking in sterile distilled water for 4 hours and then carried out in graded dilutions. With dilution series 10-1, 10-2, and 10-3. Dilution is carried out to reduce the number of bacteria suspended in the sample liquid (Hafsan, Sukmawaty E, 2016). Then, the isolation of bacteria was carried out on selective media using the spread plate method. The spread plate method, also called the spread plate method, is a method of growing bacteria by spreading a bacterial suspension onto the surface of a solid medium (agar) (Hafsan, Sukmawaty E, 2016). The media used is a selective medium intended to grow Lactic Acid Bacteria, called media de Man, Rogosa, and Sharpe Agar (MRSA) and de Man, Rogosa, and Sharpe Broth (MRSB) media. The two media only slightly differ in intended use, whereas de Man, Rogosa, and Sharpe Agar (MRSA) are used to isolate and grow LAB due to solid media. At the same time, de Man, Rogosa, and Sharpe Broth (MRSB) is a liquid medium that is used to make it easier for LAB to produce and release bacteriocins in the liquid media. It can also be used as a medium for storing cultures (stock culture) (Ningsih et al. al., 2018). The results of isolation using the spread plate method obtained 3 isolates, but only 1 isolate was detected as LAB. Then, inoculation was also carried out on the only LAB isolate. Inoculation is a technique of transferring one isolate (culture) from another isolate, which is carried out from old media to new media to obtain a pure culture and separate the isolate from other unwanted microorganism contamination (Romadhon Badaring et al., 2020). Inoculation was carried out from old de Man, Rogosa, and Sharpe Agar (MRSA) media to new de Man, Rogosa, and Sharpe Agar (MRSA) media using the T-streak method. The T-streak method also called the T-streak method, is a zig-zag scratch method in each quadrant, with three quadrants in one agar media container. The scratch method aims to isolate one colony of microorganisms from other mixed colonies or inoculate or rejuvenate isolates from old media into new media (Hafsan, Sukmawaty E, 2016). The isolates obtained in the isolation and inoculation of LAB from water used to soak Arabica coffee cherries on Mount Sindoro are as follows.

Table 1 . Results of morphological observations on LAB colony isolates.

Isolate Code	Colony Morphological Characteristics					Colony Surface
	Form	Size	Color	edge	Elevation Angle	
X1	Circular (Round)	Small	Milk white	Fine	Convex	Shiny



Figure 1 . Isolate in quadrant 3.

Source: Personal documents)

Microscopic Detection and Physiological Testing

Microscopic detection is carried out with the help of a microscope because the object of observation is so small that it cannot be seen with the sense of sight without aids. Microscopic detection of bacteria can help determine the shape of the bacterial cell itself. In addition, it is generally carried out in conjunction with physiological tests on bacterial cells, such as the Gram stain test and spore staining. However, other physiological tests, such as the motility test, can be performed macroscopically.

Gram staining is one of the staining techniques for microorganisms to be studied. Gram staining is a differential staining technique, namely a staining technique that generally uses more than one type of substance solution or dye reagent, which functions to show the differences between microorganism cells. The Gram staining technique causes the division of bacteria into two groups, namely the Gram-positive bacteria group and the Gram-negative bacteria group. The Gram-positive bacteria group is a group of bacteria that can retain crystal violet reagent when exposed to alcohol, even at a concentration of 95%; the Gram-positive bacteria group shows a blue-purple color. Meanwhile, the Gram-negative bacteria group cannot retain the crystal violet reagent. Hence, the blue-purple color fades when exposed to alcohol, so the Gram-negative bacteria show a red-pink color because they are given the safranin reagent. This can happen because there are differences in the composition and structure of the cell wall between the two groups of bacteria. Where the cell wall structure of the Gram-negative bacteria group is thinner, and the peptidoglycan content is low, even though the composition of lipids or fats or the like is higher. Because the cell wall structure of the Gram-negative group is thinner, the lipid composition is easily extracted, thereby increasing the fading of the crystal violet reagent during Gram-C treatment.

In contrast to the Gram-positive bacteria group, the lipid composition is lower. However, the cell walls become hydrated with a lot of peptidoglycan content. When Gram C is treated with a reaction, the pore size decreases, causing the crystal violet reagent to be retained (Wardani et al., 2022). The following is a picture of the results of Gram staining on the isolates obtained.

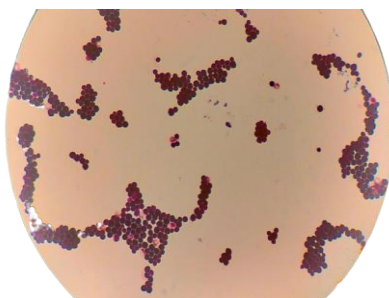
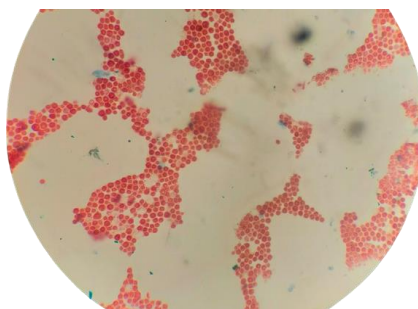


Figure 2. Appearance of isolates, including groups of Gram-positive bacteria.

Source: Personal documents

Spore staining is also a differential staining technique. Spore staining aims to differentiate between endospores and vegetative cells. Endospores, without coloring reagents, can still be seen under a microscope with a round, transparent shape and are very refractile. However, with the spore staining technique using the commonly used method - the Schaeffer-Fulton method, it will be easier to differentiate between endospores and vegetative cells where the main coloring reagent for staining spores is 5% malachite green, which can enter the spores by heating. When the malachite green reagent colors bacterial cells (vegetative cells), the bacteria with endospores will bind to the

reagent so that it does not fade when rinsed with distilled water. At the same time, the reagent will wash off from the vegetative cells. Thus, bacterial cells that have endospores will be green or bluish-green (Hafsan, Sukmawaty E, 2016). Isolates that are LAB will not show the presence of spores in the cells (Bell et al., 2005). Therefore, BAL isolates will show a red-pink color when observed. This is due to the main reagent fading from the vegetative cells, then being treated with the safranin reagent, the safranin will color the vegetative cells. Of course, safranin cannot affect the color that has been bound by endospores (Hafsan, Sukmawaty E, 2016). The following is a picture of the isolate obtained from the spore staining results.



Picture 3. The appearance of the isolate includes vegetative cells.

Source: Personal documents

The motility test aims to determine the motile or non-motile nature of the movement of bacteria. The movement of bacteria is influenced by the presence or absence of flagella in bacteria. Motile bacteria certainly have flagella to help with movement (motility) (Usman et al., 2015). Motile bacteria are characterized by the spread of bacterial growth throughout the media so that the media will appear more turbid due to the spread of bacterial motility. In contrast, non-motile bacteria are characterized by bacterial growth that does not spread, only growing in the puncture zone at the time of inoculation and presence. Bacteria in the zone will appear more clearly (Yuwita, 2021). The following is a picture of the isolates obtained from the motility tests.

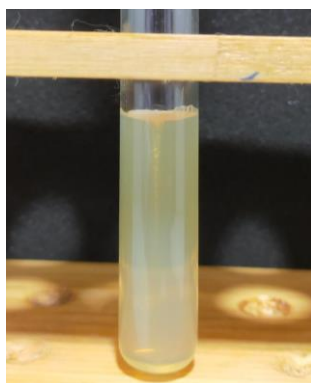


Figure 4. The appearance of the isolate is non-motile.

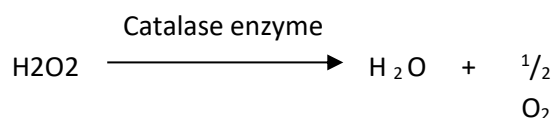
Source: Personal documents

Biochemical Reaction Testing

The biochemical reaction test was carried out to know the biochemical reaction of the research object, in this case, bacteria. Biochemical reaction tests can be carried out with simple and complex reagents to determine the ability of bacteria to produce catalase enzymes, ferment sugar, and use substrates for growth.

The catalase test is a test performed to determine the presence or absence of the catalase enzyme in the tested bacteria. The catalase test was carried out by cultivating bacterial isolates on Tryptic Soy Agar (TSA) media with the aim of enrichment of the isolates so that the isolates were able to be in optimal conditions when tested, especially BAL, which has fastidious properties. Therefore, isolate cultivation and direct testing on Tryptic Soy Agar (TSA) media because this media

is generally used to cultivate and isolate bacteria with fastidious or nonfastidious properties (Robinson, 2014). This test uses a 3% hydrogen peroxide (H₂O₂) solution, which is dropped directly onto the test isolate. The test results were declared positive (having the catalase enzyme) as indicated by bubbles forming shortly after H₂O₂ was dropped. These bubbles are oxygen (O₂) (Putra, 2010). The formation of O₂ bubbles cannot be separated from the mechanism of breaking down the hydrogen peroxide compound into water and oxygen (Aisyah et al., 2014), or the breakdown scheme is as follows.



However, the results of this study did not produce O₂ bubbles, which means that the tested bacterial isolates did not produce the catalase enzyme. The book Bergey's Manual of Systemic Bacteriology (p. 464) states that, generally, the LAB group does not produce the catalase enzyme. However, several strains in several species of the LAB group can break down hydrogen peroxide by pseudo catalyse or catalase when it contains heme compound complexes.



Figure 5. The appearance of the isolate does not produce O₂ bubbles.

Source: Personal documents

The citrate test was carried out using Simmon's Citrate Agar (SCA) on slanted media and aimed to determine the ability to use citrate as the only source of carbon and energy by bacteria (Kurniawan et al., 2023). This can happen because the SCA media contains bromthymol blue reagent, which functions as an indicator of changes in pH values resulting from biochemical reactions by bacteria. The bromthymol blue reagent at pH 6.9 is green, but if the pH increases to 7.6 (alkaline), the color will change to blue. This color change occurs due to using acid from the media to make it alkaline. However, the isolates obtained in this study showed no color change to blue, as shown in the image below.



Figure 6. Appearance of isolates without citric (acid) use.

Source: Personal documents

The Triple Sugar Iron (TSI) test was carried out using slanted Triple Sugar Iron Agar (TSIA) media containing three types of sugar (glucose, lactose, and sucrose), ferric citrate, and phenol red. Apart from that, inoculation of isolates in this test was carried out using zig-zag strokes, which aimed to determine whether the bacteria could ferment the three types of sugar, and also carried out with vertical punctures to the bottom of the media, which aimed to determine whether the bacteria could form hydrogen sulfide (H_2S) from the hydraulic reaction of the sulfur-containing amino acid cysteine and producing H_2 and CO_2 gas from the fermentation reaction. Because it contains phenol red reagent, this media is red and will change color to yellow because the bacteria produce acid from their ability to ferment one, two, or three types of sugar (depending on the part of the media that changes color). In contrast, the media will remain red if no acid is formed. Also, if the fermentation produces by-products, the butt of the media will crack or even break from the bacteria inoculated by puncture. Then, because it contains ferric acid, it will show the formation of a black precipitate if the bacteria can desulfurase the amino acid cysteine to hydrogen sulfide (H_2S).

Meanwhile, the isolate obtained shows that all parts of the media (slant and butt) change color to yellow. Apart from that, it shows that there is no formation of black precipitate and no cracks in the butt of the media, which means that the bacterial isolate does not produce H_2S , H_2 gas, and CO_2 . Briefly, it can be summarized as shown in Table 2 below.

Table 2. Results of observations of the biochemical reactions of isolates to the Triple Sugar Iron (TSI) test.

Isolate Code	<i>Triple Sugar Iron (TSI) Test</i>					
	Glucose	Lactose	sucrose	H_2S	H_2	CO_2
X1	+	+	+	-	-	-
Note:	All parts of the media turn yellow.			No black precipitate is formed.	There were no cracks or even cracks on the <i>butt</i> media.	



Figure 7. Appearance of the isolate's biochemical reaction results to the TSI test.
Source: Personal documents

The Methyl Red-Voges Proskauer (MR-VP) test was carried out using the same composition because it is based on the ability of bacteria to react with dextrose (glucose) after being given the reagent for each test between the MR and VP tests. The Methyl Red (MR) test aims to determine the ability of bacterial fermentation of glucose to produce mixed acids through the heterofermentative pathway. In contrast, the Voges Proskauer (VP) test aims to determine whether or not the ability of bacteria to ferment carbohydrates into 2,3 butanediol (Suarjana et al., 2017) in the MR test using methyl red reagent, which is a positive indicator if the color of the media turns red (can produce mixed acids from the reaction of carbohydrate fermentation) and a negative indicator if the media turns yellow. Meanwhile, the VP test uses 40% KOH reagent and α -naphthol, which can determine whether there is production of acetyl-methyl carbonyl (acetoin) - the leading compound in the 2,3 butanediol synthesis reaction. The 40% KOH reagent can convert acetoin into diacetyl so that a red color is formed, and α -naphthol functions to clarify the red color formed so that the VP test is declared positive. The VP test is negative if the media changes color to cloudy yellow. In this study, the isolate showed a color change to yellow in the Methyl Red (MR) test and a color change to cloudy yellow in the Voges Proskauer (VP) test, so it can be summarized as shown in Table 3 below.

Table 3. Results of observations of the biochemical reactions of isolates to Methyl Red-Voges Proskauer (MR-VP) test.

Isolate Code	Methyl Red Test (MR)	Voges Proskauer test (VP)
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X1

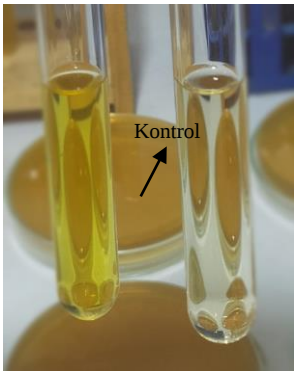


Figure 8. MR color changes to yellow.
Source: Personal documents

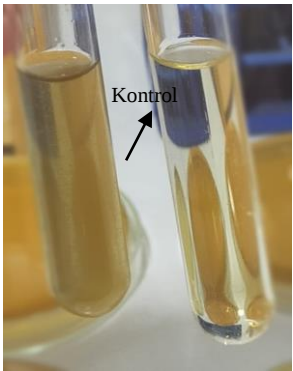


Figure 9. Change of VP color to cloudy yellow.
Source: Personal documents

The mannitol test used Mannitol Salt Agar (MSA) media containing mannitol sugar. Mannitol is a hydrogenated sugar from β -fructose. With the help of the phenol red reagent which makes the media red, the bacteria that have the ability to ferment sugar in the MSA media will change color to yellow due to acid production which occurs and even increases (Hayati et al., 2019). Meanwhile, the results of the mannitol test on the isolates obtained in this study were negative, which is indicated by the media remaining red, as shown in Figure 8 below.



Figure 8. The appearance of the media does not change color.

Source: Personal documents

Detection of Lactic Acid Bacteria (LAB) Groups

Observations made from morphological appearance, physiological testing, and biochemical reactions can help in detecting groups of test microorganisms down to the genus level. This research only focused on the Lactic Acid Bacteria (LAB) group. This research has also proven that there is 1 isolate which is confirmed to be LAB which can be isolated directly from its natural environment, namely the outer skin of Arabica coffee cherries (*C.arabica*), through soaking for 4 hours.

The isolates obtained were Gram-positive with a cocci (round/spherical) shape. Some were slightly elongated to look like an ovoid shape composed of single cells, double cells (pairs), and even cells with long and short chains. In addition, this isolate is a vegetative cell that does not produce spores and is a non-flagellate bacterium, so it is non-motile. Then, according to the biochemical reaction, the isolate cannot react with hydrogen peroxide (H_2O_2) because it does not have the catalase enzyme, does not use citrate as a source of carbon and energy, and is unable to form hydrogen sulfide (H_2S), as well as hydrogen gas (H_2) and carbon dioxide (CO_2) from the results of fermenting sugars (glucose, lactose, and sucrose) thus indicating that the isolate has a homolactic fermentation metabolism (homofermentative); because it has a homofermentative metabolism, it cannot ferment glucose into mixed acids and does not produce acetyl-methyl carbonyl (acetoin) so it cannot ferment glucose into 2,3 butanediol, and cannot ferment mannitol.

Based on the determination contained in the book Bergey's Manual of Determinative Bacteriology, Seventh Edition and supported by the book Bergey's Manual of Systemic Bacteriology, Second Edition, Volume Three: The Firmicutes and the book The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, Volume II, the isolate has the same morphology, physiology, and biochemistry as *Lactococcus* sp. which grows on the surface of the agar to form small colonies with a diameter of less than 1 mm, white in color which forms a smooth circle (round); which has spherical cells or elongated oval cells like ovoids with a series of short chains or pairs, some cultures form long chains; including Gram positive; does not form endospores; non-motile; facultative anaerobes; catalase negative; mesophilic (grows at temperatures between 10-40°C); acid

production from glucose, maltose, and lactose; can sometimes ferment xylose, arabinose, sucrose, trehalose, mannitol, and salicin; Carbohydrate fermentation is homofermentative; The main end product of glucose fermentation is L(+)-lactic acid; optimal growth at pH values close to neutral; including the lactic acid bacteria group of the Streptococcaceae family; natural habitat for plants.

Thus, based on data from ITIS (Integrated et al.), Schleifer classified *Lactococcus* sp. as follows.

kingdom	: Bacteria
Subkingdom	: Posibacteria
phylum	: Firmicutes
Class	: Bacilli
Order	: Lactobacillales
Family	: Streptococcaceae
Genus	: Lactococcus

CONCLUSION

The research "Detection of Lactic Acid Bacteria (LAB) in Water Used to Soak Arabica Coffee Cherries on Mount Sindoro" was able to prove that there are natural Lactic Acid Bacteria (LAB) on the skin of Arabica coffee cherries cultivated at an altitude of approximately 2000 meters above sea level on Mount Sindoro, Temanggung, which was isolated from water used to soak Arabica coffee for 4 hours. LAB is included in the group of lactic acid bacteria from the family Streptococcaceae, genus *Lactococcus*, which is shown in the results of macroscopic detection through observing colony morphology (shape, color, and size of colonies), microscopic detection through physiological tests (Gram staining, spore staining, and motility test), and biochemical reaction tests (catalase test, citrate test, Triple Sugar Iron (TSI) test, Methyl Red-Voges Proskauer (MR-VP) test, and mannitol test).

REFERENCES

- Aisyah, A., Kusdiyantini, E., & Supriyadi, A. (2014). Isolasi, karakterisasi bakteri asam laktat, dan analisis proksimat dari pangan fermentasi "tempoyak." *Jurnal Akademika Biologi*, 3(2), 31–39.
- BPS, B. (2020). Badan pusat statistik. *Direktorat Jendral Peternakan Dan Kesehatan Hewan, Kementan*.
- Dalimunthe, H., Mardhatilah, D., & Ulfah, M. (2021). Modifikasi proses pengolahan kopi arabika menggunakan metode honey process. *Jurnal Teknik Pertanian Lampung (Journal of Agricultural Engineering)*, 10(3), 317–326. <http://dx.doi.org/10.23960/jtep-l.v10i3.317-326>
- Dewi, H. (2014). *Isolasi bakteri asam laktat dan pemanfaatannya pada fermentasi pati sagu*.
- Firmansyah, A., Wardhana, D. I., & Setiawan, A. P. (2023). Pengaruh Suhu Dan Variasi Waktu Pengeringan Terhadap Uji Organoleptik Teh Cascara. *Seminar Nasional Fakultas Pertanian*, 6(1).
- Hafsan, Sukmawaty E, M. M. (2016). *Penuntun Praktikum Mikrobiologi*. Universitas Islam Negeri Alauddin.
- Hayati, L. N., Tyasningsih, W., Praja, R. N., Chusniati, S., Yunita, M. N., & Wibawati, P. A. (2019). Isolasi dan identifikasi *Staphylococcus aureus* pada susu kambing peranakan etawah penderita mastitis subklinis di Kelurahan Kalipuro, Banyuwangi. *Jurnal Medik Veteriner*, 2(2), 76–82.
- Kurniawan, R. K., Darniati, D., Abrar, M., Fakhrurrazi, F., Jalaluddin, M., & Erina, E. (2023). Isolasi Dan Identifikasi Bakteri *Eschericia Coli* Pada Produk Ceker Ayam Bakar Di Gampong Ulee Lheue Kota Banda Aceh. *JURNAL ILMIAH MAHASISWA VETERINER*, 7(2).

- Ningsih, N. P., Sari, R., & Apridamayanti, P. (2018). Optimasi Aktivitas Bakteriosin yang Dihasilkan oleh *Lactobacillus brevis* dari Es Pisang Ijo. *Jurnal Pendidikan Informatika Dan Sains*, 7(2), 233–242.
- Puspaningrum, L., Yuwono, S. S., & Martati, E. (2018). Karakteristik fisikokimia dan sensoris fruit leather apel manalagi (*malus sylvestris* mill) dengan substitusi pisang candi (*musa paradisiaca*). *Jurnal Teknologi Pertanian*, 19(3), 173–182. <https://doi.org/10.21776/ub.jtp.2018.019.03.4>
- Putra, A. M. (2010). Analisis produktifitas gas hidrogen dan gas oksigen pada elektrolisis larutan KOH. *Jurnal Neutrino: Jurnal Fisika Dan Aplikasinya*. <https://doi.org/10.18860/neu.v0i0.1642>
- Putri, A. L., & Kusdiyantini, E. (2018). Isolasi dan identifikasi bakteri asam laktat dari pangan fermentasi berbasis ikan (Inasua) yang diperjualbelikan di Maluku-Indonesia. *Jurnal Biologi Tropika*, 1(2), 6–12. <https://doi.org/10.14710/jbt.1.2.6-12>
- Robinson, R. K. (2014). *Encyclopedia of food microbiology*. Academic press.
- Romadhon Badaring, D., Muhammad, F., & Bahri, A. (2020). *Identifikasi Morfologi Mikroba Pada Ruang Water Closet Jurusan Biologi Universitas Negeri Makassar*.
- Suarjana, I., Ketut, G., Besung, I. N. K., & Hapsari Mahatmi, K. T. P. G. (2017). Modul isolasi dan identifikasi bakteri. *Bali: Universita Udayana*.
- Sujaya, I. N., Nocianitri, K. A., Aryantini, N. P. D., Nursini, W., Ramona, Y., Orikasa, Y., Kenji, F., Urashima, T., & Oda, Y. (2016). Identifikasi dan Karakterisasi Bakteri Asam Laktat Isolat Susu Segar Sapi Bali. *Jurnal Veteriner Juni*, 17(2), 155–167.
- Temanggun, P. K. (2014). *Kebijakan Umum dan Program Pembangunan Daerah Kabupaten Temanggung Tahun 2013-2018*. RPJMD: Kabupaten Temanggung.
- Usman, D., Supriyadi, A., & Kusdiyantini, E. (2015). Fermentasi kopi robusta (*Coffea canephora*) menggunakan isolat bakteri asam laktat dari feces luwak dengan perlakuan lama waktu inkubasi. *Jurnal Akademika Biologi*, 4(3), 31–40.
- Wardani, K. A., Sakati, S. N., Sulami, N., Syahrir, M., & Kanan, M. (2022). *Teori Mikrobiologi*. Yayasan Penerbit Muhammad Zaini.
- Yuwita, F. (2021). *Potensi Bakteri Endofit Pada Biji Mangrove:(Sonneratia alba) Sebagai Antibakteri Staphylococcus aureus dan Escherichia coli*. UPT. Perpustakaan.



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