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ANTIBACTERIAL ACTIVITY OF BACTERIAL PIGMENT EXTRACTS ISOLATED FROM FRUIT AND VEGETABLE WASTE AGAINST *Staphylococcus epidermidis*

AKTIVITAS ANTIBAKTERI EKSTRAK PIGMEN BAKTERI HASIL ISOLASI DARI LIMBAH BUAH DAN SAYUR TERHADAP BAKTERI *Staphylococcus epidermidis*

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Abstract

Acne affects approximately 9.4% of the global population and become one of the big eight skin diseases due to *Staphylococcus epidermidis* infection. This infection can be treated using bacterial pigments for their potential activities as antioxidant, anticancer, and antimicrobial with low toxicity and stable productivity. In this study, pigments were harvested and purified from pigment-producing bacteria which were isolated from fruit and vegetable waste, and the antibacterial activity was conducted with disc diffusion method against *S. epidermidis*. There were three pigment-producing isolates (LBS 6, LBS 12, and LBS 14) that produced green pigments with antibacterial activity against *S. epidermidis*. Among the pigments produced by the three isolates, pigments from LBS 14 had the widest zone of inhibition and the strongest antibacterial activity followed by LBS 6 and LBS 12 respectively. In addition, through Two-Way ANOVA analysis, it was found that there was a significant effect on the utilization of pigments from different bacterial isolates and variations in concentration on the diameter of the inhibition zone as well as the interaction between them.

Keywords: Antibacterial; Bacterial pigment; Fruit and vegetable waste; *Staphylococcus epidermidis*

Abstrak

Jerawat terjadi pada sekitar 9,4% dari populasi global dan merupakan salah satu penyakit kulit yang paling umum, sering kali disebabkan oleh infeksi Staphylococcus epidermidis. Infeksi ini dapat diobati menggunakan pigmen bakteri yang telah terbukti memiliki aktivitas potensial sebagai antioksidan, antikanker, serta antimikroba dengan toksisitas rendah dan produktivitas yang stabil. Dalam penelitian ini, pigmen diekstrak dan dimurnikan dari bakteri penghasil pigmen yang diisolasi dari limbah buah dan sayur, kemudian aktivitas antibakterinya diuji dengan metode difusi cakram terhadap S. epidermidis. Terdapat tiga isolat penghasil pigmen (LBS 6, LBS 12, dan LBS 14) yang menghasilkan pigmen hijau dengan aktivitas antibakteri terhadap S. epidermidis. Di antara pigmen yang diproduksi oleh ketiga isolat, pigmen dari LBS 14 menunjukkan zona hambat paling luas dan aktivitas antibakteri paling kuat, diikuti oleh LBS 6 dan LBS 12. Selain itu, melalui analisis Two-Way ANOVA, ditemukan adanya pengaruh yang signifikan antara pemanfaatan pigmen dari isolat bakteri yang berbeda dan variasi konsentrasi terhadap diameter zona hambat, serta interaksi di antara keduanya.

***Kata Kunci**: Antibakteri; Limbah buah dan sayur; Pigmen bakteri; Staphylococcus epidermidis*

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INTRODUCTION

Dermatitis is one of the most common diseases in Indonesia (Purba & Rotua, 2022) and acne is one of the most common dermatitis. Acne ranks eighth as a skin disease with a prevalence reaching 9.4% in the global population (Vasam et al., 2023) due to *Staphylococcus epidermidis* (Purba & Rotua, 2022). *S. epidermidis* is a gram-positive, coccus-shaped bacterium with a cell diameter of around 0.8–1.0 µ (Purba & Rotua, 2022). *S. epidermidis* together with *Propionibacterium acne* and *Staphylococcus aureus* are bacteria that cause acne by infecting the skin and causing blocked oil ducts (Dewi et al., 2018). In acne, *S. epidermidis* will irritate the blocked follicle area, so that an abscess is formed which then swells and bursts, resulting in inflammation spreading to the skin tissue (Retnaningsih et al., 2019).

Generally, bacterial infections can be overcome by using antibiotics, especially antibacterial agents that can interfere with the growth and metabolism of certain bacteria. Antibacterial compounds can be produced by several living organisms as secondary metabolites (Pelczar & Chan, 2006; Wiguna et al., 2016; Khristnaviera & Meitiniarti, 2017). One of them is a natural pigment produced by plants, animals, or microorganisms (de Medeiros et al., 2022). Among other natural pigments, pigments produced by bacteria are currently popular for their low toxicity, stable production, and can be quickly harvested. Thus, several pigments produced by bacteria are known to have antioxidant, anticancer, and antimicrobial activities (Usman et al., 2017).

Pigment-producing bacteria can be isolated and purified from various sources or materials in the environment. One of them is fruit and vegetable waste as an important source of natural pigments not only because they naturally contain extractable pigments but also because they can be used as fermentation substrates for pigment production by some microorganisms (Di Salvo et al., 2023). Therefore, this research was carried out to determine the antibacterial potential of pigments produced by bacteria isolated from fruits and vegetable waste against *S. epidermidis*.

MATERIALS AND METHODS

Isolation of Pigment Producing Bacteria from Fruits and Vegetables Waste

Fruit and vegetable waste was collected from one of the traditional markets in Yogyakarta, Indonesia. They were washed thoroughly and blended until smooth then put into a 500 mL duran bottle and followed with serial dilution method from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, until 10^{-5}. Samples from each dilution were inoculated on Nutrient Agar (NA) medium using the pour plate method and incubated at 37 °C for 5–7 days. Bacterial colonies that produce pigment are then transferred to a fresh medium as a colony library. Isolates that have strong pigmentation are purified and made into stock on a slanted NA medium.

Bacterial Screening Based on Growth Rate

The purified pigment-producing bacterial isolates were then grown in Nutrient Broth (NB) and incubated in an incubator shaker at 30 °C and 150 rpm. The Optical Density (OD) value of the isolate was measured using UV-vis spectrophotometry with a wavelength of 600 nm at 0, 4, 8, 20, 24, 28, 40, 44, and 48 hours post-incubation. From the measurement results, a growth curve was created to determine the exponential and stationary phases of each bacterial isolate. The growth rate of bacterial isolates was calculated by dividing the OD value during the exponential period by the time of the exponential phase. The growth rate (µ) is obtained by dividing the natural logarithm (*ln*) of the optical density value during the exponential phase (OD_2 - OD_1) with the time (t_2 - t_1) of the exponential phase (Widdel, 2007). Three isolates that had equivalent or equal growth rates were selected for pigment production.

Bacterial Pigment Production and Extraction

The selected bacterial isolates were inoculated on Mueller Hinton Agar (MHA) medium which had been added with 2% glycerol in Erlenmeyer using a cotton swab and incubated at 37 °C for 3–5 days until the pigment was visible. The bacterial culture was added with 50 mL of Muller Hinton Broth (MHB) with 2% glycerol followed by incubation in an incubator shaker at 37 °C and 150 rpm

for 72 hours. The bacterial culture suspension was harvested and transferred to a 50 mL conical tube for centrifugation at 9,500 rpm for 10 minutes to separate the cells from the supernatant. The supernatant containing pigment was then transferred to another Erlenmeyer flask and mixed with 70% methanol in a ratio of (1:1). The suspension was then dried using a rotary evaporator and oven at 50 °C for 5–7 days until a dry extract was obtained.

Antibacterial Activity Assay`

The dry pigment extract was dissolved in DMSO in concentrations of 5; 7.5; and 10% (w/v). The antibacterial activity assay was carried out against *S. epidermidis* through the disc diffusion method. Antibiotic Amoxicillin trihydrate 0.05% (w/v) was used as a positive control and DMSO 0.05% (w/v) was as a negative one.

Analysis of Antibacterial Activity Results

The data was analyzed with Two-Way Analysis of Variance (ANOVA) to determine whether there was a significant effect of administering fruit and vegetable waste bacterial pigment extracts on *S. epidermidis* with antibiotic amoxicillin trihydrate as a comparative positive control.

GC-MS Analysis

The dry pigment extract was dissolved in methanol, and then 1 µL of the sample was injected into the GC-MS with a glass column 30 m long and 0.25 mm in diameter. The oven temperature was set at 50 °C (isothermal for 1 minute) with an increase of 5 °C/minute to 250 °C (isothermal for 3 minutes), then increased 10 °C/minute to 280 °C (isothermal for 5 minutes). The separation rate was 10 mL/min with a separation ratio of 10:1. The sample run was carried out for 61 minutes. The recorded masses of compounds and fragments were matched with NIST 8 to identify the compounds present in the sample.

Characterization of Pigment-Producing Bacterial Isolates

Characterization of bacterial isolates was carried out based on Bergey's Manual Determination of Bacteriology (Holt et al., 1994) by observing colony and cell morphology and physiological tests.

RESULTS

Isolation of Pigment-Producing Bacteria

Pigment-producing bacteria have been successfully isolated from fruit and vegetable waste. The results of bacterial isolation from these wastes showed that the isolate samples at a 10^{-2} dilution had several colonies that were not too dense, with the most varied growth of pigment-producing bacteria (Figure 1).

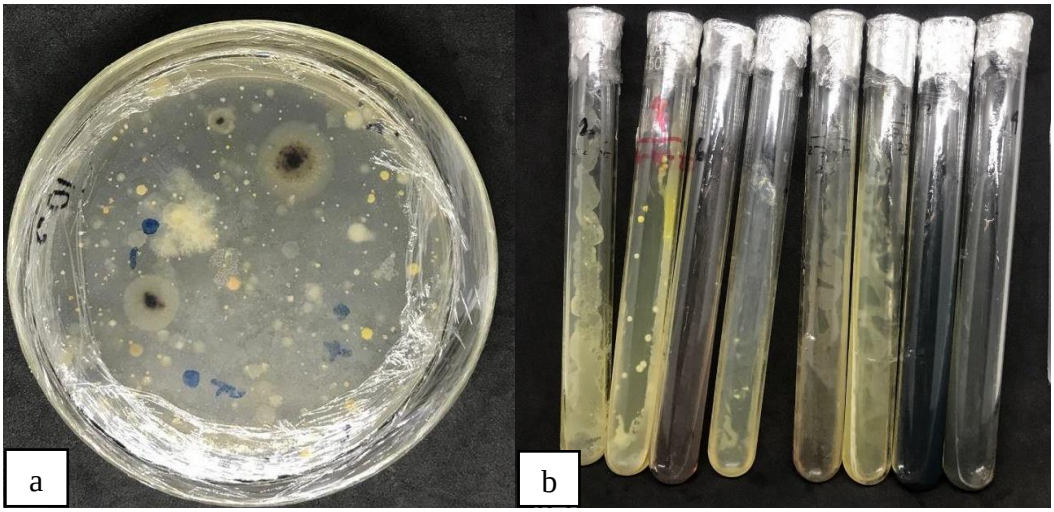

Figure 1. Bacterial colonies from fruit and vegetable waste on Nutrient Agar medium. Isolate sample of 10^{-2} dilution with the most diverse colonies of pigment-producing bacteria (a) and pure isolate of pigment-producing bacteria from fruit and vegetable waste (b)

This indicates that the 10^{-2} dilution offered the best combination of colony separation and visibility, facilitating more accurate strain identification and differentiation that produces pigment. The range of pigments found in the isolates suggests the existence of several bacterial species, each of which may have its specific metabolic route for producing pigments. This variety may be useful for future research on the synthesis of natural pigment and its possible uses in the food, cosmetics, and pharmaceutical industries. Among the bacterial colonies, eight of them were selected and purified as they have strong pigmentation (LBS 2, LBS 4, LBS 6, LBS 8, LBS 9, LBS 11, LBS 12, and LBS 14).

Growth Rate of Pigment-Producing Bacteria from Fruit and Vegetable Waste

The growth rate of bacterial isolates LBS 2, LBS 4, LBS 6, LBS 8, LBS 9, LBS 11, LBS 12, and LBS 14 was measured based on the OD value at the time of incubation. The following is a growth curve of bacterial isolates from fruit and vegetable waste (Figure 2). This curve illustrates the varying growth dynamics of each isolate, highlighting differences in their ability to proliferate under the same conditions. Such variability in growth rates suggests that these isolates may have distinct metabolic capabilities or adaptations to the nutrient composition of fruit and vegetable waste.

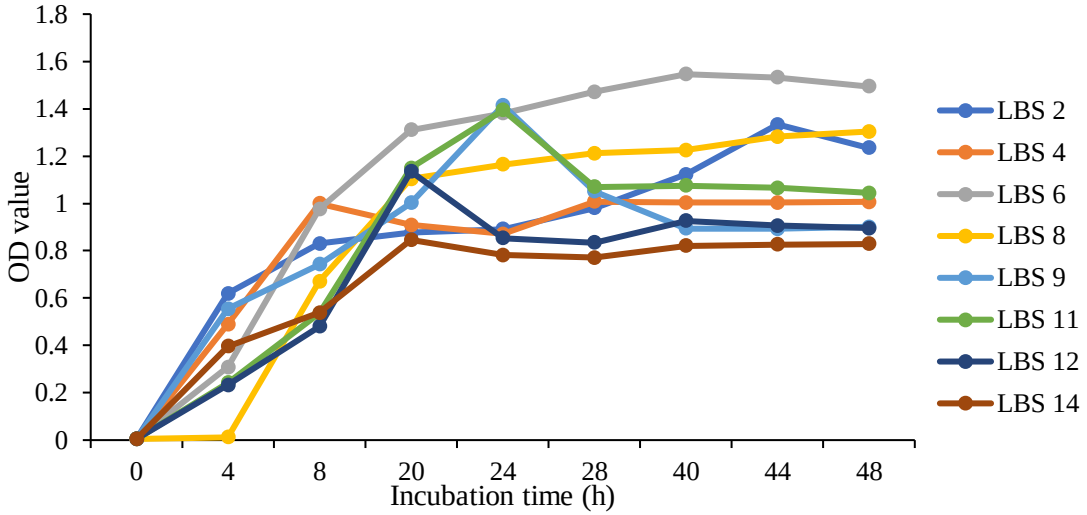

Figure 2. Growth curve of pigment-producing bacteria (LBS 2, LBS 4, LBS 6, LBS 8, LBS 9, LBS 11, LBS 12, and LBS 14) that were isolated from fruit and vegetable waste

The bacterial growth rate is then calculated based on the OD value at the beginning and end of the exponential phase. The results of the growth rate calculation are presented in Table 1. The growth rate varies significantly across the different isolates, with some showing rapid growth (e.g., LBS 4) and others displaying more moderate or stable growth patterns (e.g., LBS 8 to LBS 14). This variation could be due to differences in the growth characteristics or environmental adaptability of each isolate.

Table 1. The growth rate of fruit and vegetable waste bacterial isolates

Isolate name	μ
LBS 2	0.13
LBS 4	0.66
LBS 6	0.28
LBS 8	0.34
LBS 9	0.24
LBS 11	0.23
LBS 12	0.27
LBS 14	0.26

Note: μ= bacterial growth rate

The growth rates of the bacterial isolates (Table 1) show significant variability, with LBS 4 exhibiting the highest growth rate at 0.66, indicating a rapid proliferation compared to the other isolates. In contrast, LBS 2 has the lowest growth rate at 0.13, suggesting a much slower growth pace.

The remaining isolates, LBS 6 through LBS 14, display moderate growth rates ranging from 0.23 to 0.34, with only slight differences between them. This suggests that while LBS 4 grows much faster, the other isolates maintain a more consistent and stable growth rate. These differences in growth rates could be attributed to the varying metabolic efficiencies or environmental adaptations of the isolates, influencing their ability to thrive under similar conditions. Three isolates that have almost equal growth rates (LBS 6, LBS 12, and LBS 14) were then tested for antibacterial activity using the disc diffusion method

Antibacterial Activity

The antibacterial activity test of the pigment extract produced by the bacterial isolates LBS 6, LBS 12, and LBS 14 was carried out using the disc diffusion method and resulted in clear zones as an indicator of bacterial growth inhibition (Figure 3). These clear zones surrounding the discs indicate that the pigment extracts have strong antibacterial qualities and successfully stop the tested *S. epidermidis* from growing. The inhibitory zone sizes differed between the isolates, suggesting that the pigment extracts' antibacterial activity was not all the same. These results demonstrate the potential of these bacterial pigments as bioactive chemicals with potential uses in the development of novel antibacterial drugs. Further studies could explore the chemical nature of the pigments responsible for this activity, as well as their efficacy against a broader spectrum of bacteria.

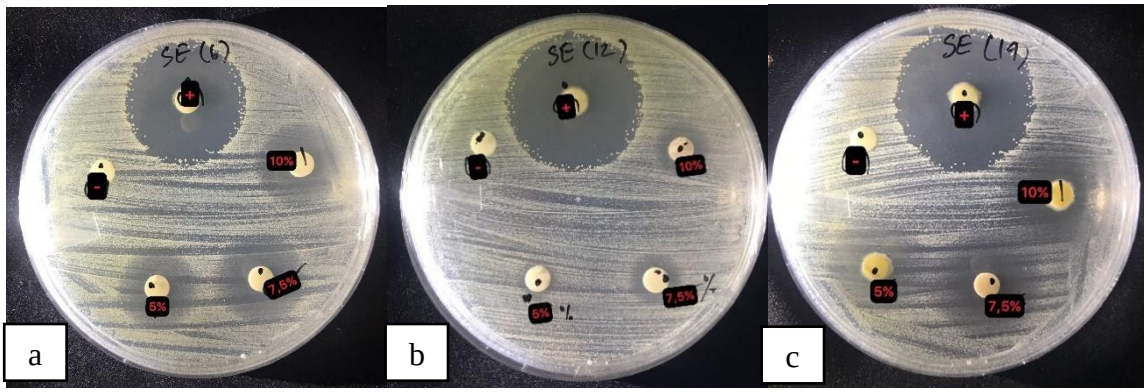

Figure 3. Inhibition zone that formed during antibacterial activity test of bacterial isolate pigments LBS 6 (a), LBS 12 (b), and LBS 14 (c) against *Staphylococcus epidermidis*

The diameter of the resulting inhibition zone around the disc as in Figure 3 was measured and analyzed using Two-Way ANOVA. The area of the inhibition zone (mm) is shown in Table 2.

Table 2. Diameter of inhibition zones of pigments produced by bacterial isolates against *Staphylococcus epidermidis*

Isolate	Pigment concentration	Inhibition zone (mm)		
		1st Attempt	2nd Attempt	Average ± SD
LBS 6	5%	11.7	11.4	11.55 ± 0.62
	7.5%	14.3	14.6	14.45 ± 0.62
	10%	14.3	16.5	15.40 ± 0.62
	Positive control	23.4	26	24.70 ± 0.62
	Negative control	0	0	0
LBS 12	5%	1.8	2.7	2.25 ± 0.62
	7.5%	7	6	6.50 ± 0.62
	10%	8	6.5	7.25 ± 0.62
	Positive control	24.9	25.6	25.25 ± 0.62
	Negative control	0	0	0
LBS 14	5%	15.3	14.9	15.10 ± 0.62
	7.5%	16.7	17.2	16.95 ± 0.62
	10%	18.7	19.2	18.95 ± 0.62
	Positive control	25.5	24.3	24.90 ± 0.62
	Negative control	0	0	0

Note: Positive control= amoxicillin trihydrate 0.05%; Negative control= DMSO 0.05%

The data suggests that the pigment extracts from LBS 14 have the strongest antibacterial properties, followed by LBS 6, while LBS 12 is the least effective. The antibacterial impact depends on concentration, with greater inhibition zones occurring at higher doses. These results demonstrate the pigment extracts potential as all-natural antibacterial agents, with the effectiveness of these extracts varied according to the isolate and concentration. Subsequent investigations may delve deeper into the particular substances accountable for the antimicrobial efficaciousness and their possible uses in healthcare or commercial settings.

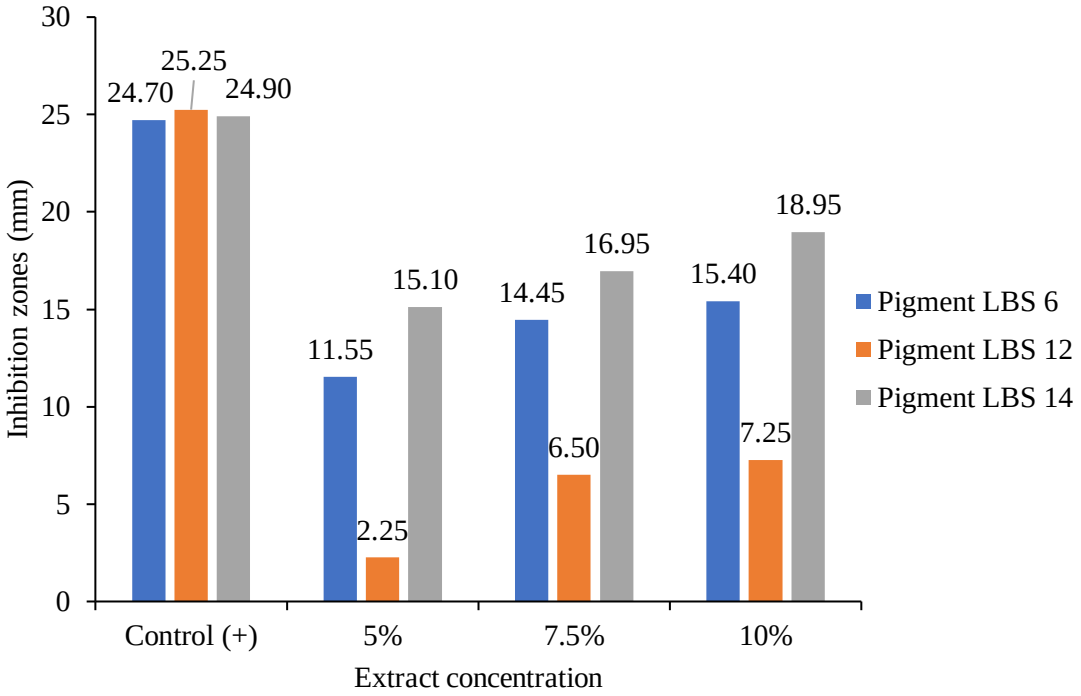

Figure 4. Diagram of antibacterial test results for bacterial pigments LBS 6, LBS 12, and LBS 14 against *Staphylococcus epidermidis*. Note: Control += antibiotic amoxicillin trihydrate 0.05%

In Figure 4, there are noticeable differences in the diameter of the inhibition zone formed from the pigment produced by the bacterial isolates LBS 6, LBS 12, LBS 14, and the control on the growth of *S. epidermidis*. The pigment produced from LBS 14 has the widest zone of inhibition at all concentrations, indicating a stronger antimicrobial activity compared to the other isolates. This suggests that LBS 14 may possess unique or more potent bioactive compounds responsible for inhibiting the growth of *S. epidermidis*. The narrower inhibition zones observed with LBS 6 and LBS 12, as well as the control, further emphasize the superior efficacy of the pigment from LBS 14.

GC-MS Analysis

Bacterial pigments that have antibacterial activity are analyzed using GC-MS to determine their compound profile. This analysis provides a detailed breakdown of the chemical constituents present in the pigments, helping to identify the specific compounds responsible for their antimicrobial properties. The compounds of each bacterial pigment can be seen in Table 3, where the variations in chemical profiles among the different isolates are evident.

Table 3. Compound content in pigment extracts LBS 6, LBS 12, and LBS 14 GC-MS analysis results

Compound	Pigment	Molecular formula	Molecular weight	Group
2,4-Pentanedione, 3-(1-methyl ethyl)-	LBS 6 & LBS 12	$C_8H_{14}O_2$	142.099	Ketones
1,3,3-Trimethoxybutane	LBS 6	$C_7H_{16}O_3$	148.110	Ethers
Octane	LBS 12	C_8H_{18}	114.141	Alkanes
Decane	LBS 6	$C_{10}H_{22}$	142.172	Alkanes
n-Hexyl acrylate	LBS 14	$C_9H_{16}O_2$	156.115	Esters

Cyclo(L-prolyl-L-valine)	LBS 6 & LBS 14	$C_{10}H_{16}N_2O_2$	196.121	Cyclic dipeptides
Octahydrodipyrrolo[1,2-a:1',2'-d]pyrazine5,10-dione-,(5aR,10aR) (isomer 1)	LBS 6	$C_{10}H_{14}N_2O_2$	194.106	Heterocyclic
Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione	LBS 14	$C_{11}H_{18}N_2O_2$	210.137	Pyrrolopyrazines
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro3-(2-methylpropyl)-	LBS 14	$C_{11}H_{18}N_2O_2$	210.137	Pyrrolopyrazines

It is known that several compounds contained in the pigments produced by bacterial isolates LBS 6, LBS 12, and LBS 14 are compounds from the alkane, ketone, ether, esther, cyclic dipeptide, heterocyclic, and pyrrolopyrazine groups (Table 3). It is known that all bacterial pigments have more than different compounds.

Characterization of Pigment-Producing Bacteria

Morphological and physiological characterization was carried out on pigment-producing bacterial isolates LBS 6, LBS 12, and LBS 14. This analysis included examining the shape, size, color, and texture of the bacterial colonies, as well as assessing their growth patterns under different environmental conditions such as temperature, pH, and salinity. The morphological and physiological characteristics of the bacteria are shown in Table 4, providing a comparative overview of their distinct traits. These characteristics not only help in the identification and classification of bacterial isolates but also offer insights into their adaptability and potential.

Table 4. Morphological and physiological characteristics of LBS 6, LBS 12, and LBS 14 bacterial isolates

Characteristic	Character type	Isolate		
		LBS 6	LBS 12	LBS 14
Colony morphology	Colony form	Circular	Circular	Circular
	Colony edge	Undulate	Entire	Entire
	Elevation	Convex	Convex	Convex
	Surface	Smooth	Smooth	Smooth
	Pigment	Green	Green	Green
Cell morphology	Cell shape	Bacil	Coccus	Coccus
	Gram	Negative	Positive	Positive
	Endospores	No	No	No
Physiology	Catalase	Positive	Positive	Positive
	Mannitol	Negative	Positive	Positive
	Glucose	Negative	Negative	Negative
	Fructose	Negative	Positive	Positive
	Lactose	Negative	Negative	Negative
	Nitrate	Negative	Negative	Negative

Note: Positive characters for catalase indicate the bacteria have the catalase enzyme, while negative characters indicate the bacteria don't have the catalase enzyme; positive characters for mannitol, glucose, fructose, and lactose indicate that bacteria can ferment these carbohydrates, while negative characters indicate that bacteria can't ferment these types of carbohydrates; the positive character for nitrate indicates that bacteria can reduce nitrate, while the negative character indicates that bacteria can't reduce nitrate

DISCUSSION

Pigment-producing bacteria were successfully isolated from fruit and vegetable waste. This finding promotes the production of sustainable bioproducts and waste reduction in addition to helping in the search for new natural pigments (Rukmana & Zulaika, 2017). Among the bacterial isolates, four were selected as their different pigment colors: yellow, cream, and green. Bacterial pigment formation is a complex process driven by the need to persist, adapt, and grow in a variety of

environments. Depending on the type of bacteria and the habitat in which they reside, these pigments' particular uses and advantages can differ greatly (Numan et al., 2018; Agarwal et al., 2023).

The growth rate of these bacterial isolates was then measured based on the Optical Density (OD) value during the incubation time to obtain the chart shown in Figure 2. From the curve, it can be determined the exponential and the stationary phase. The exponential phase is the phase where the fastest growth occurs and is shown by the OD value continuing to increase dramatically until a certain time so that the curve on the graph tends to form a vertical line. Meanwhile, the stationary phase is the phase when growth has stopped, and the OD value has not increased significantly or tends to remain constant so that the graph curve tends to form a horizontal line (Maier & Pepper, 2015). The bacterial growth rate is then calculated based on the OD value at the beginning and end of the exponential phase and the time at which the exponential phase occurs. From the results of the growth rates of fruit and vegetable waste bacteria presented in Table 2, it is known that LBS 6, LBS 12, and LBS 14 have almost equivalent growth rates that are 0.28; 0.27; and 0.26.

Antimicrobial assay of the extracted pigments from LBS 6, LBS 12, and LBS 14 against *S. epidermidis* at concentrations of 5; 7.5; 10%, showed strong inhibitory activity. Meanwhile, pigment samples from LBS 12 at a concentration of 5% had weak inhibitory activity and at concentrations of 7.5% and 10% had moderate inhibitory activity. The inhibition zone grouping is carried out based on the diameter of the resulting inhibition zone. Inhibition zones with a diameter of ≤5 mm are considered to have weak bacterial inhibition activity, inhibition zones with a diameter of 5–10 mm are considered to have moderate inhibition activity, inhibition zones with a diameter of 11–20 mm are considered to have strong inhibition activity, and inhibition zones with a diameter ≥20 mm are considered to have very strong inhibitory activity (David & Stout, 1971). These inhibitions were still comparable to 0.05% amoxicillin trihydrate as positive control which had very strong inhibitory power. Amoxicillin is an antibiotic that is known to have the ability to kill gram-negative and gram-positive bacteria by damaging their cell walls. In contrast, 0.05% DMSO as negative control didn't produce an inhibition zone at all (Tabel 2) as their non-toxic effect on bacteria (Sagita & Pratama, 2020; Pratiwi, 2008).

Based on the Two-Way ANOVA test, it is known that the significance value of the three pigments produced by different bacteria and their respective concentrations is $P < 0.05$, so it can be interpreted that there is a real difference in the use of pigments produced by different bacteria and different concentrations towards the diameter of inhibition zone. Apart from that, it is also known that there is an interaction between the use of different pigments and variations in concentration in determining the results of the inhibition zone or antibacterial activity against *Staphylococcus epidermidis*.

In this study, to investigate the bioactive compound in the pigment, analysis with GC-MS was also carried out on the pigments produced by LBS 6, LBS 12, and LBS 14. It was shown that the pigments LBS 6 and LBS 14 are thought to contain the same bioactive compounds, Cyclo (L-prolyl-L-valine) (Tabel 2) which are known to have antibacterial activity against *S. aureus* and *Micrococcus luteus* (de Carvalho & Abraham, 2012). Cyclo (L-prolyl-L-valine) is also known to inhibit quorum-sensing from pathogenic bacteria where this compound will interfere with signaling from certain receptors so that the response given is disrupted. Through this mechanism, quorum-sensing inhibitors will influence several biological processes in pathogens, such as inhibiting the swarming process, disrupting motility, and inhibiting biofilm formation. Most quorum-sensing inhibitor compounds can cause the death of microorganisms at higher concentrations (Kapadia et al., 2022). Meanwhile, the LBS 12 pigment is not known to contain antibacterial compounds through GC-MS results. This may occur because the antibacterial compounds in the LBS 12 pigment extract are at very low concentrations, so they are not detected by GC-MS. This is by the results of the inhibition zone from the previous antibacterial activity test which showed that the LBS 12 pigment produced the smallest inhibition zone compared to the other two pigments.

Based on the characterization results presented in Table 3, it was confirmed that LBS 6 is a gram-negative bacteria with bacillus-shaped cells. These bacteria also known as non-spore-forming bacteria gave a positive result on the catalase test. This isolate also could not reduce nitrate, and could

not ferment mannitol, glucose, fructose, or lactose. According to the characters observed and referring to Bergey's Manual Determination of Bacteriology (Holt et al., 1994), LBS 6 was thought to be a member of the genus *Pseudomonas*. Most members of the genus *Pseudomonas* are also known to be aerobic bacteria that are widespread in nature and some species can produce green pigment (Holt et al., 1994). Meanwhile, LBS 12 and LBS 14 were known to be gram-positive bacteria with coccus-shaped cells and non-spore-forming. The two bacterial isolates also showed positive results in the catalase test, could ferment mannitol and fructose, but could not ferment glucose and lactose and could not reduce nitrate. Based on the dichotomous key, the two bacterial isolates are thought to be members of the genus *Staphylococcus*. Members of the genus *Staphylococcus* are also known to be facultative anaerobic bacteria that grow optimally at temperatures of 30–37 °C. These bacteria are often found on the skin, food products, dust, and water. Some strains of members of the species can also produce extracellular toxins and greenish pigments (Holt et al., 1994; Zemelman & Longeri, 1965).

CONCLUSION

Green pigments produced by pigment-producing bacteria isolated from fruit and vegetable waste, LBS 6, LBS 12, and LBS 14 were known to have antibacterial activity against *Staphylococcus epidermidis*. The pigment produced by LBS 14 has the widest inhibition zone so it has the most potential as an antibacterial agent compared to the other two pigments.

This pigment produced by bacteria isolated from fruit and vegetable waste has great potential to be used as a new antibacterial agent. Therefore, further research and development is needed regarding the properties of the pigments tested as antibacterial agents so that they can be utilized optimally in the future.

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