Exploration of Antibiotic Producing Bacteria from the Human Beard

Nurfazira
Department of Biology, Faculty of Mathematics and Natural Science and Health
Universitas Muhammadiyah Riau
Pekanbaru, Indonesia
150202022@student.umri.ac.id

Israwati Harahap
Department of Biology, Faculty of Mathematics and Natural Science and Health
Universitas Muhammadiyah Riau
Pekanbaru, Indonesia
israwatiharahap@umri.ac.id

Elsie
Department of Biology, Faculty of Mathematics and Natural Science and Health
Universitas Muhammadiyah Riau
Pekanbaru, Indonesia
elsie@umri.ac.id

***Abstract*-This study aims to explore antibiotic producing bacteria from beards, test antibacterial activity on *Escherichia coli* and *Staphylococcus aureus* and characterize antibiotic producing bacteria from beards. Bacteria were isolated using the pour plate method, and then tested for antibacterial activity on *E. coli* and *S. aureus* using the disk method. After that, the morphology of each bacterial colony was observed macroscopically as well as microscopically, and biochemical tests were performed. A total of 9 bacterial isolates were successfully isolated from beards. Antibacterial activity test results from 9 isolates reveal that 6 isolates were obtained to be able to inhibit *S. aureus*, namely isolates Jg 1, Jg 2, Jg 3, Jg 4, Jg 5 and Jg 8. One isolate was able to inhibit the growth of *E. coli*, namely Jg 5 isolate. Bacterial isolates from beards which have antibacterial activity were identified using the Bergey's Manual of Determinative Bacteriology identification book and 3 genera of bacteria were obtained, namely *Micrococcus*, *Planococcus* and *Staphylococcus*.**

Keywords: Beard, Antibiotic, Escherichia coli, Staphylococcus aureus.

I. INTRODUCTION

Antibiotics are chemical compounds produced by microorganisms or produced synthetically that can kill, inhibit bacteria and other organisms (Munaf & Chaidir, 1994). At this time the problem of antibiotics and their resistance is of concern to the whole world. WHO even set the theme of Antimicrobacterial Resistance and its Global Spread to commemorate World Health Day (Utami, 2011). Many antibiotics are no longer able to deal with a disease caused by a microorganism, and this happens because the ability of antibiotics to overcome or prevent infectious diseases causes their use to experience an extraordinary increase (WHO, 2015).

The increase of inappropriate use of antibiotics can cause resistance to pathogenic microbes (Sjahurrahman et al., 1999). Various ways have been done to explore new antibiotic sources with low toxicity, including by isolating antibiotic-producing bacteria from the soil (Panagan, 2011), the sea (Sunaryanto et al., 2009), plant tissue *Ocimum basilicum* L. (Utami et al., 2017); *Oryza sativa* L (Mariati, 2013) and *Vetiveria zizanioides* (Putri, 2018). Apart from land, sea and plants, a search for new sources of antibiotics has been found including beard. This was proven by Wakeam et al. (2014), who conducted a study of 408 medical workers in hospitals by taking a beard sample using the swab method and obtained results as much as 41% of men who have beards have bacteria that can inhibit the bacteria *Staphylococci coagulase-negative resistant-methylindex* and *S. aureus* which is a pathogenic bacteria. This is what drives the search for new antibiotics (bioactive compounds), one of which is by exploring bacteria that have the potential to produce antibiotics from beard.

II. METHODS

This research was conducted from May to July 2019. The beard samples were taken from 10 people whose jobs are as students, educators and education staff who work at Universitas Muhammadiyah Riau. Sample analysis was carried out at the Fish and Environmental Health Laboratory of the Central Fish Seed Center (BBIS) Sei Tibun, Kampar District, Technical Implementation

Unit (UPT) of Aquaculture, Agency of Maritime Affairs and Fisheries of Riau Province.

Tools and Materials

The tools used in this study were Laminar Air Flow (LAF), oven (Drying oven), incubator (Memmert), autoclave (My life MA 635), binocular microscope. The material used in this study was a beard sample. Media of Nutrient Agar (NA), Nutrient Broth (NB), Media of Sugar (glucose, lactose, maltose, mannitol and sucrose), Media of Methyl Red-Voges Proskauer (MR-VP), Media of Triple Sugar Iron Agar (TSIA), Media of Fermentative Oxidative (OF), Media of Sulfide Indol Motility (SIM), Media of Simons Citrate Agar (SCA), hydrogen peroxide, filter paper, NaCl, 70% alcohol, aquades and spritus. The test bacteria used were *S. aureus* and *E. coli* (culture collections were obtained from the Microbiology Laboratory of Medical Faculty, Riau University).

Bacterial Isolation

A beard sample was taken as much as 1 gr and crushed, then put into a test tube that has contained 9 ml of NaCl solution, then divortex for 2 minutes, do a gradual dilution to 10^{-5} dilution. As much as 1 ml sample from a 10^{-3}, 10^{-4} and 10^{-5} was taken and grown on NA media by pour plate, then incubated at 25-28ºC for 24 hours.

Bacterial Purification

Colonies of growing bacteria were then taken and scratched onto the surface of the NA medium, incubated at 25-28^{o}C for 24 hours. Pure isolates are marked by separate colonies. The bacterial growth was observed macroscopically including color, size, shape, border, surface, and colony elevation.

Making Bacteria Suspension Test

One bacterial loop was inoculated into a test tube containing 5 ml of NB media and incubated for 24 hours at 37 ^{o}C. The suspension of the bacteria was diluted using sterile distilled water until the turbidity was equivalent to the standard Mc.Farland I solution (liquid culture whose turbidity was equivalent to 0.5 Mc.Farland I had a population of $1x10^{7}$CFU/ml-$1x10^{8}$ CFU/ml) (Sutton, 2011).

Antibacterial Activity Test

Antibacterial activity Test was carried out using the disc method. Test bacteria that were 24 hours old were taken with a cotton swab. Furthermore, the cotton swab was dipped into the test tube and pressed gently on the wall of the test tube so that the cotton swab was not too wet, then rubbed horizontally on the NA media. The disc paper was immersed in a beard bacterial suspension, then the disc paper was placed on the NA media which already contains the test bacteria and incubated at 37 ^{o}C. Inhibition zones formed around the paper disc after 24 hours were observed. The inhibition zone is the clear zone that forms around the disk. The inhibition zone formed around the disc was then measured in diameter. The inhibition zone diameter was obtained by measuring the difference in the diameter of the inhibition zone (mm) to the diameter of the disc.

Characterization of Bacteria from Beard

Bacterial isolates that have antibacterial activity on *E. coli* and *S. aureus* were further characterized microscopically (Gram staining) and biochemical tests. Characterization carried out by means of biochemical tests include, Catalase Test, Oxidase Test, Motility Test, Citrate Test, TSIA Test, Sugar Test (Glucose, Sucrose, Lactose, Maltose and Manitol), MR Test, VP Test and OF Test.

III. RESULTS AND DISCUSSION

Results of Bacterial Isolation from Beard

The results of bacterial isolation from beards obtained 9 different bacterial isolates based on macroscopic observation. Macroscopic observations include different colors, sizes, shapes, elevations, edges and colony surfaces. Macroscopic morphological observations of 9 bacterial isolates from beards can be seen in Table 4.1.

Table 4.1. Macroscopic observation of bacterial isolate from beard

Isolate code	Color	Size	Shape	Elevation	Margin	Surface
Jg 1	Yellow	Small	Circular	Raised	Entire	Smooth glossy
Jg 2	White	Small	Circular	Raised	Entire	Smooth glossy
Jg 3	Cream	Medium	Circular	Raised	Entire	Smooth glossy
Jg 4	Cream	Small	Circular	Raised	Entire	Smooth glossy
Jg 5	White	Medium	Circular	Convex	Entire	Smooth glossy
Jg 6	Cream	Medium	Circular	Umbonate	Entire	Smooth glossy
Jg 7	Chocolate	Big	Circular	Raised	Undulate	Smooth glossy
Jg 8	White	Medium	Circular	Raised	Entire	Smooth glossy
Jg 9	Chocolate	Medium	Circular	Convex	Entire	Smooth glossy

Based on Table 4.1, the morphology of the bacterial colonies from the beard obtained has a different colony color or pigment including, the Jg 1 colony isolates are yellow; Jg 2, Jg 5 and Jg 8 are white; Jg 3, Jg 4 and Jg 6 are cream colored and isolate Jg 7 and Jg 8 are brown. Colony elevation in isolates Jg 5 and Jg 9 is convex shaped like a drop of water (convex), Jg 6 isolate has elevation with convex shape in the middle more prominent (umbonate) and the elevation of the colonies Jg 1, Jg 2, Jg 3, Jg 4, Jg 7 and Jg 8, the altitude is noticeable but flat on the entire surface (raised). Isolate Jg 7 has undulated edges, while isolates Jg 1, Jg 2, Jg 3, Jg 4, Jg 5, Jg 6, Jg 8 and Jg 9 have flat colony edges. All bacterial isolates from beards have rounded colony shape (circular) and smooth glossy surface. Morphology of bacterial isolates colonies found in beard bacterial isolates is in accordance with the statement of Cappucino & Sherman (1987) that in general the form of bacterial colonies is circular, irregular, filamentous, and rhizoid. Elevation is in the form of raised, convex, flat, umbonate and crateriform. The edges are in the form of entire, undulate, lobate, curled and filiform.

Antibacterial Activity Test

Antibacterial activity test results on 9 bacterial isolates obtained 6 bacterial isolates that can inhibit the growth of *S. aureus* and 1 isolate capable of inhibiting *E. coli*. This is marked by the formation of clear zones around the disc paper (Table 4.2).

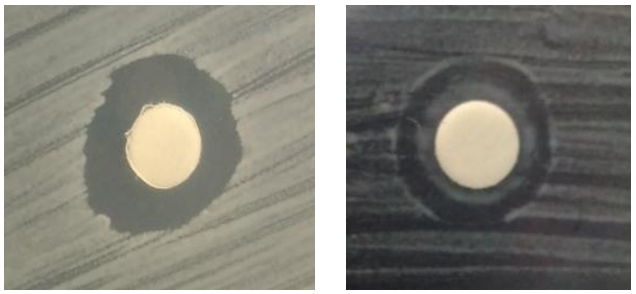
Table 4.2 Antibacterial activity test for bacterial isolate from beard

No	Inhibitory Zone Diameter				
	Isolate Code	Staphylococcus aureus	Category	Escherichia coli	Category
1	Jg 1	7 mm	Moderate	-	-
2	Jg 2	4 mm	Weak	-	-
3	Jg 3	3,5 mm	Weak	-	-
4	Jg 4	4,5 mm	Weak	-	-
5	Jg 5	3,5 mm	Weak	6,5 mm	Moderate
6	Jg 6	-	-	-	-
7	Jg 7	-	-	-	-
8	Jg 8	6 mm	Moderate	-	-
9	Jg 9	7 mm	-	-	-

Note : (-) No inhibition zone

Based on Table 4.2, inhibition zones produced by beard bacterial isolates on S. aureus, there are 4 bacterial isolates that are categorized as weak namely Jg 2, Jg 3, Jg 4, and Jg 5 isolates; 2 bacterial isolates were medium category, Jg 1 and Jg 8 isolates; 3 bacterial isolates that did not produce inhibition zones namely isolates Jg 6, Jg 7 and Jg 9. Meanwhile, inhibition zones produced by beard bacterial isolates on E. coli there were 1 isolate with medium category namely Jg 5 isolates; 8 isolates that did not produce inhibition zones namely isolates Jg 1, Jg 2, Jg 3, Jg 4, Jg 6, Jg 7, Jg 8 and Jg 9. Based on the calculation of the inhibition zone diameter observed on the media, the inhibition zone can be categorized as follows, diameters> 20 mm are categorized as very strong, 11-20 mm are categorized as strong, 6-10 mm are categorized as moderate and <5 mm are categorized as small (Susanto et al., 2012).

The biggest inhibition zone produced by beard bacteria on *S. aureus* was Jg 1 isolate with a diameter of 7 mm and the smallest inhibition zone is in isolates Jg 3 and Jg 5 with a diameter of 3.5 mm. The largest inhibitory zone on *E. coli* was produced by Jg 5 isolate with a diameter of 6.5 mm (Figure 4.1).



(a) (b)

Figure 4.1 Antibacterial activity of beard isolates Jg 1 bacteria on *S. aureus* (a) and Antibacterial activity of beard isolates Jg 5 on *E. coli* (b)

One of the beard bacterial isolates, jg 5 isolates, is thought to have potential as antibiotic producing bacteria. This is marked by the formation of a clear zone (inhibitory zone) around the disc paper in the two target bacteria, namely *E. coli* and *S. aureus*. According to Wahjudhi *et al*. (2014), the formation of clear zones caused by these microorganisms maintain their presence in the habitat by removing secondary metabolite compounds (antibacterial) which will be diffused around them, so that other microorganisms that are in the vicinity cannot grow. The formation of a clear zone or zone of inhibition indicates that there is an emphasis on the growth of microorganisms by antibacterial compounds.

Antibacterial compound testing was carried out on two target bacteria representing groups of Gram positive and Gram negative bacteria. The results of the antibacterial activity test of beard bacteria isolates in inhibiting the negative gram bacteria (*E. coli*) were less compared to Gram positive bacteria (*S. aureus*). Fitri & Bustam (2010) said that Gram-negative bacteria have better protection on antimicrobial compounds compared to Gram-positive bacteria because they have different cell wall components. Gram-positive bacterial cell walls are relatively thin because they are only composed by peptidoglycans compared to Gram-negative bacterial cell walls that are not only composed by peptidoglycan but are also composed by lipoproteins, outer membranes and lipopolysaccharides. The structure of the cell wall allows metabolite substances produced by beard bacterial isolates to be unable to enter in Gram negative bacteria.

Microscopic Characterization and Biochemical Test of Antibiotic Producing Bacteria from Beard

Beard bacterial isolates that have inhibition zones on *E. coli* and *S. aureus* are then subjected to biochemical tests and Gram staining of bacterial isolates (Table 4.3).

Table 4.3 Biochemical test of antibiotic producing bacteria from beard

No	Biochemical Test	Isolate Code					
		Jg 1	**Jg 2**	**Jg 3**	**Jg 4**	**Jg 5**	**Jg 8**
1	Catalase	+	+	+	+	+	+
2	Oxidase	+	-	-	-	-	-
3	Motility	-	+	+	-	-	+
4	TSIA	-	-	-	-	-	-
5	OF	F	F	O	O	F	F
6	Citric	-	-	-	-	-	-
7	MR	-	-	-	-	-	-
8	VP	-	+	+	+	+	-
9	Glucose	-	-	-	+	-	+
10	Sucrose	-	-	-	-	-	-
11	Lactose	-	-	-	-	-	-
13	Maltosa	-	-	-	-	-	+
14	Manitol	-	-	-	-	-	-
Microscopic Observation							
15	Gram	+	+	+	+	+	+
16	Cell shape	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus
	Genus	Micrococcus	Planococcus	Planococcus	Staphylococcus	Staphylococcus	Planococcus
		Oxidative					

Note: (+) : Positive; (-) : Negative**; (**F) : Fermentative**; (**O) :

Gram staining results and biochemical tests that have been carried out are in

accordance with the book Bergey's Manual of Determinative Bacteriology, where isolate Jg 1 is a genus of *Micrococcus*. According to Majeed (2017), carotenoid pigments produced by *Micrococcus* bacteria have antibacterial and antifungal activity and can absorb ultraviolet (UV) rays ranging from 300-500 nm, so that the pigment of the genus *Micrococcus* is widely used in medicine, cosmetics and as a protective agent against sunlight. *Micrococcus luteus* is a species of the genus *Micrococcus* which produces carotenoid pigments.

Bacterial isolates from beards namely Jg 2, Jg 3 and Jg 6 are genera of *Planococcus*. Holt *et al*. (1994) stated that bacteria from the genus *Planococcus* have a round cell shape with a diameter of 1.0-1.2 µm, Gram positive, has one or two flagella per cell. Aerobic bacteria, positive catalase and negative oxidase. The optimum temperature for growth of *Planococcus* bacteria is 27-37 ^{o}C. The most habitats of these bacteria are in the sea. Meanwhile, bacterial isolates from beard Jg 4 and Jg 5 are genera of *Staphylococcus*. Based on research by Hong *et al*. (2014), *Saphylococcus* bacteria can produce bacteriocin, an antimicrobial peptide synthesized by ribosomes. Antimicrobial substances produced by these bacteria are widely applied for alternative antibiotics in the agricultural and food industries.

IV. CONCLUSION

Based on the results of research conducted, it can be concluded that 9 isolates of bacteria isolated from beards were obtained. Six isolates were able to inhibit the growth of *S. aureus* bacteria and 1 isolate was able to inhibit the growth of *E. coli* bacteria. The identification results of 6 beard bacterial isolates that have antibacterial activity obtained by 3 genera, namely: *Micrococcus*, *Planococcus* and *Staphylococcus*.

ACKNOWLEDGEMENT

Special thanks to the Ministry of Research, Technology and Higher Education for providing research funding through the 2019 Research Scheme of Student Creativity Program (PKM-P) grant, so that this research can be carried out well.

REFERENCES

1. Munaf, S., and Chaidir, J. 1994. Obat antimikroba. farmakologi UNSRI. EGC. Jakarta.
2. Utami, E., R. 2011. Antibiotika, Resistensi, Dan Rasionalitas Terapi. Fakultas Sains dan Teknologi UIN Malik Ibrahim. Malang. Vol.(1) 4: 191-198.
3. WorldHealth Organization. 2015. Antimicrobial Resistance. http://www.who.int/mediacentre/factsheet/fs194/en/. Diakses pada tanggal 22 April 2018
4. Sjahrurachman A., W. Kumala dan Nurjadi, T. 1999. Kepekaan Kuman Terhadap Antibiotika Golongan Kuinolon dan Sefalosporin. CDK 124. p. 17-20.
5. Sutton, S. 2011. *Determination of Inuculum for Microbiological Testing*. Summer Vol. 15 Number 3.
6. Cappucino, J.G. and Sherman, N. 1987. *Microbiology*: *A Laboratory Manual*. The Benjamin Cummings Publishing Company Inc. California USA.
7. Susanto, D., Sudrajat dan Ruga, R. 2012. Studi Kandungan Bahan Aktif tumbuhan Meranti Merah (*Shorea leprosula Miq*) Sebagai Sumber Senyawa Antibakteri. 11(2):1-5.
8. Wahjudi, M., Algadrie, L., dan Chrisnasari, R. 2014. Isolasi Bakteri dari Tanah Gunung Kapur dan Pengujian Aktivitas Antibakteri Isolat Terhadap Bakteri *Esherichia coli* dan *Staphylococcus aureus. Jurnal imiah sains & teknologi.* (7): 7-17.
9. Fitri, L. dan Bustam, B., M. 2010. Screening of Antimicrobial Producing Strains Isolated From the Soil of Grassland Rhizosphere in Pocut Meurah Intan Forest Park, Seulawah, Aceh Besar. *Biodiversitas*. 11(3): 255-261.
10. Holt, J G, Krieg N R, Sneath, P, H, A, Staley, J T and William, S T. 1994. *Bergey's Manual of Determinative Bacteriology*, Ninth Edition, William and Wilkins Company, United Stated.

11. Hong, H., Quan, L., Heu, S., Jung, K., S., Han, S., W., Moon, E dan Roh, E. 2014. A New Antimicrobial Substance Produced by *Staphylococcus pasteuri* Isolated from Vegetables. *J. Food Sci Biotechnol*. 23(3). Hal 983-990.
12. Majeed, H., Z. 2017. A New Antimicrobial Activity of *Micrococcus luteus* Cartenoid Pigment. *Journal of Science*. Vol.28
13. Panagan, A. 2011. Isolasi Mikroba Penghasil Antibiotik Dari Tanah Kampus UNSRI Indralaya Menggunakan Media Ekstrak Tanah. Program Studi Kimia. Universitas Sriwijaya. Sumatra Selatan. Vol. (14)3.
14. Putri, T., R. 2018. Isolasi Actinomycetes Endofit Dari Tanaman Akar Wangi (*Vetiveria zizanioides*) dan Uji Aktivitas Senyawa Antibakteri Terhadap *Staphylococcus aureus* dan *Escherichia coli*. Program Studi Biologi. Fakultas MIPA dan kesehatan. Universitas Muhammadiyah Riau. *Jurnal Photon.* Vol. 8.
15. Utami, P., Mahyarudin dan Mistika, Z. 2017. Isolasi, Identifikasi dan Uji Aktivitas Bakteri Endofit Daun Kemangi (*Ocimum basilicum. L*) Terhadap *Staphylococcus Aureus. Skripsi.* Program Studi Pendidikan Dokter. Fakultas Kedokteran. Universitas Tanjung Pura. Pontianak.
16. Mariati, D. 2013. Potensi Isolat *Actinomycetes* dari Rizosfer Padi *Oryza sativa L* Sebagai Penghasil Antibiotik. *Skripsi*. Fakultas Farmasi. Universitas Muhammadiyah Surakarta. Surakarta.
17. Sunaryanto, R., Marwoto, B., Irawadi, T., Mas'ud, A., Z., dan Hartoto, L. 2009. Isolasi Dan Penapisan Aktinomisetes Laut Penghasil Antimikroba. Balai Pengkajian Bioteknologi BPPT. Institut Pertanian Bogor. Jawa Barat. Vol. 14(2):98-101.
18. Wakeam, E., Hernandez, R., A., and Rivera M., D.2014. Bacterial ecology of hospital worker's facial hair: a cross-sectional study. *J Hosp infect*; 87:63-67.