Andalas University
Indonesia

2020
ICBEAU

# CERTIFICATE
## *of Achievement*

No. 047/CP/ICBEAU/XII/2020

This certificate is proudly appreciated to the article

**"Screening of Anti-MRSA Metabolites in Bacteria Symbiotic with *Batissa violaceae* celebensis Marten 1897"**

author(s):

**Sri Anggarini Rasyid***, Dheatri Wira Agustina Lena, Suryani As'ad, Upik A Miskad, Rahmawati Minhajat, and Ridwan Adi Surya

presented in

## 2nd ICBEAU

**International Conference of Bio-Based Economy for Application and Utilization**

Authorized by
Institution of Research and Community Services (LPPM)
of Andalas University
West Sumatera, Indonesia
Via Zoom Meeting on December 16th, 2020

**Dr.-Ing. Ir. UYUNG GATOT S. DINATA, M.T.**
Chairman of Institution of Research and Community Services (LPPM) of Andalas University

**Prof. Dr. sc. agr. Ir. JAMSARI, M.P.**
Chairman of Committee

*Oral Presenter

# Screening of Anti-MRSA metabolites in bacteria symbiotic with *Batissa violaceae celebensis* marten 1897



# Screening of Anti-MRSA metabolites in bacteria symbiotic with *Batissa violaceae celebensis* marten 1897

**S A Rasyid[1,2*], D W A Lena[2], S As'Ad[3], U A Miskad[4], R Minhajat,[5,6] and R A Surya[7]**

[1]Doctoral Program, Graduate School of Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[2]D-IV Medical Laboratory Technology Study Program, Mandala Waluya University
[3]Department of Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[4]Department of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[5]Department of Internal Medicine, Division of Hematology Medical Oncology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[6]Department of Histology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[7]Department of Environmental Science, Faculty of Forestry and Environmental Science Halu Oleo University Kendari Southeast Sulawesi, Indonesia

E-mail: anggarini.09@gmail.com

**Abstract**. Currently, infectious diseases are still a serious problem in Indonesia, especially with thewidespread resistance of microbes to antibiotics. *Staphylococcus aureus* as one of the commonpathogens causing infection has experienced resistance to various classes of beta-lactam antibiotics known as Methicillin-resistant Staphylococcus aureus (MRSA). Therefore antibiotic alternatives sourced from nature are needed to treat the MRSA pathogenic infection. This study aims to explore the potential of bacteria in symbiosis with Pokea Shells (*Batissaviolaceacelebensis* Martens 1897) from Konawe Regency, Southeast Sulawesi Province to be used as the newest anti-MRSA.In this research, Pokea Shell's symbiont bacteria were isolated from Konawe Regency to obtain pure isolates. Pure isolates that have been produced from secondary metabolites to be tested for their potential in inhibiting the growth of pathogenic bacteria MRSA. The inhibition test was done qualitatively by using a paper disk. Based on the results five bacterial isolates were obtained with different abilities to inhibit MRSA bacteria. Based on the qualitative test obtained that the Bvc1 and Bvc3 bacterial isolates had the potency to be used as anti-MRSA agents. These isolates have an inhibition zone of 25 mm and 22 mm with a sensitive category according to CLSI standards (Sensitive ≥17 mm). Determination of the gram of these 2 isolates found as gram- positive bacteria in the form of bacilli so it can be concluded that the 2 isolates can be used as anti-MRSA agents.

Keywords: Anti-MRSA, *Batissa violacea celebensis* Martens 1897, Screening, Symbiont bacteria

## 1. Introduction

The danger of antibiotic resistance is recognized as an ongoing serious problem both in developed and developing countries[1]. In Indonesia, it exacerbates the serious threat of infectious diseases. *Staphylococcus aureus*, one of the common pathogens causing various infections, has evolved resistance to various classes of antibiotic beta-lactam, known as *Methicillin-resistant Staphylococcus aureus* (MRSA)[2]

Infections due to *Staphylococcus aureus* are usually treated with the administration of antibiotics[3]. However, in some cases, several strains of *Staphylococcus aureus*, such as *Methicillin-resistant Staphylococcus aureus* (MRSA), were found to be resistant to antibiotics[4]. One of the worst outcomes of MRSA infection is amputation. Apart from causing wound severity, MRSA is also strongly suspected to exacerbate pneumonia (lung infection) [5].

Indonesian waters are rich in invertebrates such as Mollusca, one of which is Bivalvia or shellfish [6]. Southeast Sulawesi Province is known for its biodiversity. The evidence of it can be found in the freshwater ecosystem on the Pohara River in Konawe District. The Pohara River holds potential biological resources such as freshwater clams known as *pokea* (*Batissa violacea celebensis* Martens 1897) by local people[7]. Several studies [8]mentioned that bivalves possess a plethora of benefits on human health. According to [9], *pokea* clam extract possesses the ability to inhibit the growth of *Staphylococcus aureus*. [10] reported that jackknife clam (*Solen sp.*) and windowpane oyster (*Placuna placenta*) extracts possess antibacterial activities against *S. aureus*. [11] reported that the extract of *Atactodeastriata* clam possesses antibacterial activities against *S. aureus* and *E. coli*. [13] reported that Pokea clam can empirically treat various diseases, one of which is an infectious disease caused by microorganisms such as bacteria. Therefore, this study was conducted in the hope to identify the anti-MRSA potential of bacterial isolates that are symbiotic with *Batissa violacea celebensis* Martens 1897.

## 2. Materials and Methods

As an explorative study, this study sought to find and identify the symbiont bacteria of pokea clam (*Batissa violacea celebensis* Martens 1897) that hide the potential as an anti-MRSA. This study consisted of several stages :

### *2.1. A sampling of Pokea Clams (Batissa violacea celebensis Marten 1897).*

The source of bacterial isolates used in this study was pokea clams, which is a local endemic resource of Konawe District, Southeast Sulawesi Province. These clams were taken from the Pohara River and put into sterile plastic bags and stored in a cool box.

### *2.2. Isolation of Symbiont Bacteria*

The symbiont bacteria were isolated using the pour plate method on nutrient agar media. The bacteria association were incubated overnight at 28 $^{o}$C. *S. aureus* was inoculated on nutrient broth and was grown overnight at 27 $^{o}$C according to the shaker condition. After the incubation period, colony morphological observations were made to determine which isolates were purified by the pour plate method. The bacterial isolates obtained were purified by re-isolation to ensure the purity of the culture. The purified isolates were then stored on agar slants as isolate stocks for further testing [13].

### *2.3. Production of Bacterial Secondary Metabolite Compounds*

Secondary metabolite compounds were produced based on the phase of bacterial growth, which was the stationary phase. This process was performed by taking 10% ($10^7$ cells/mL) of bacterial culture to be inoculated into 50 mL of production medium, which was then shaker incubated in accordance with the optimum incubation time. Then, the metabolites were harvested and centrifugated at 1000 rpm for 10 minutes to obtain a metabolite compound form of supernatants (free cells) [13]

*2.4. Testing of the Secondary Metabolite Compound Against MRSA Bacteria*

The qualitative test of the antagonist test of the bacterial metabolite compound was carried out by testing the secondary metabolites of bacteria in the form of free cells. Disc paper with a diameter of 6 mm which has been inoculated with a bacterial supernatant with a cell density of $10^7$cfu/ml was placed on the surface of the agar medium that has been inoculated with MRSA bacteria with a density of $10^7$cfu / ml and then incubated at 37°C for 24 hours [14]. The diameter of the clear zone formed around the disc was calculated using a digital caliper.

## 3. Result and Discussion

The results of the test of anti-MRSA potential of bacterial isolates that were symbiotic with *Batissaviolaceacelebensis* Martens 1897 are as follows:

*3.1. Results of Symbiont Bacteria Isolation*

The isolation of symbiont bacteria was performed using the pour plate method. After the incubation period, the growing bacterial colonies were observed morphologically. The results are presented in Table 1.

**Table 1.** The Number of Bacterial Isolates That Are Symbiotic with *Batissa violacea celebensis* Martens 1897 based Differences in Colony Morphology

| No. | Isolate Code | Colony Shape | Edge | Elevation | Colour | Inner Structure |
|---|---|---|---|---|---|---|
| 1. | Bvc1 | Circular | Entire | Low Convex | Beige | Transparent |
| 2. | Bvc2 | Irregular | Undulate | Low Convex | Beige | Transparent |
| 3. | Bvc3 | Circular | Entire | Low Convex | Beige | Transparent |
| 4. | Bvc4 | Irregular | Undulate | Low Convex | Beige | Transparent |
| 5. | Bvc5 | Circular | Entire | Low Convex | Beige | Transparent |

*3.2. Results of Inhibition Test against MRSA*

The bacterial inhibition test was carried out by using the disc diffusion method. The results are presented in the following table 2 and figures 1 and 2:

**Table 2.** Inhibition Ability of Bacteria That Are Symbiotic with *Batissa violacea celebensis* Martens 1897

| No. | Isolate Code | Inhibition Zone Area (mm) | Description* |
|---|---|---|---|
| 1. | Bvc1 | 25 | Sensitive |
| 2. | Bvc2 | - | |
| 3. | Bvc3 | 22 | Sensitive |
| 4. | Bvc4 | - | |
| 5. | Bvc5 | - | |
| 6. | Control + | 17 | Sensitive |
| 7. | Control - | - | |

*Resistant (≤13 mm), Intermediate (14-16 mm), Sensitive (≥17 mm)
(Standard Clinical and Laboratory Standards Institute(CLSI)

Based on the results of a qualitative screening test, two bacterial isolates were produced which had inhibitory activity against MRSA pathogenic bacteria and the two isolates were used for the next test stage. The five isolates that possessed the highest inhibitory activity were Bvc1 (25 mm) and Bvc3 (22 mm). Isolate Bvc1 and Bvc3 had a wider inhibition zone than the positive control (penicillin). Penicillin is a methicillin class of antibiotics that are already resisted by *Staphylococcus aureus.* One form of antibiotic resistance by *Staphylococcus aureus* bacteria is resistant to penicillin class antibiotics[15].

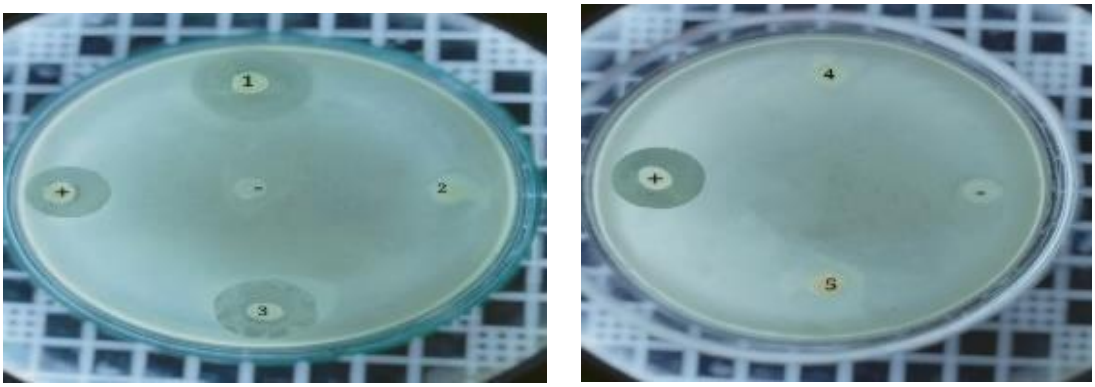


**Figure 1.** The results of the Test of Anti-MRSA Potential of Bacterial Isolates That Were Symbiotic with *Batissaviolaceacelebensis* Martens 1897

*3.3. Characteristic Results of Bacterial Isolate Cells with Potential Anti-MRSA*

Bacterial isolates that had inhibition zones were characterized by Gram staining. The characteristic results of bacterial isolate cells with potential anti-MRSA are shown in the following figure.

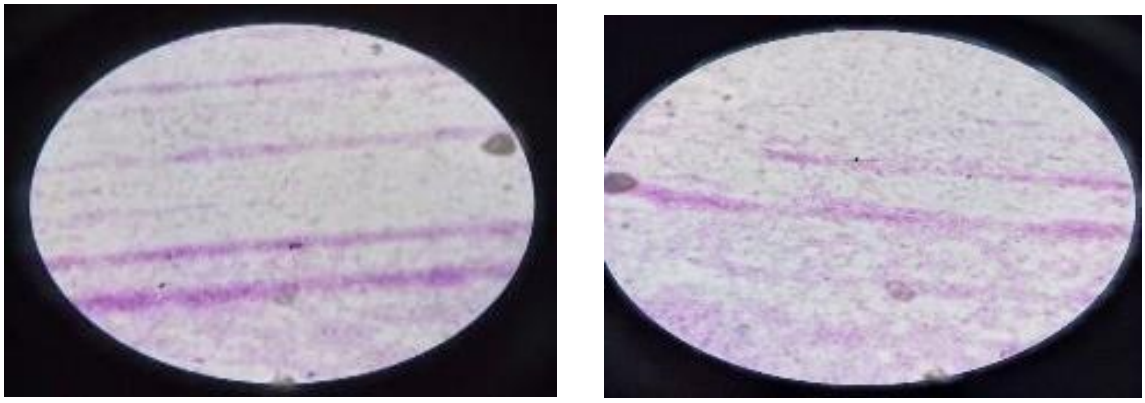

**Figure 2.** The Results of the Bacterial Isolate Staining Test That Were Symbiotic with *Batissaviolaceacelebensis* Martens 1897 and Had the Potential as an Anti-MRSA

## 4. Discussion

Based on the results of bacterial isolation using nutrient agar media and observation of colony morphological characters, 5 (five) bacterial isolates that were symbiotic with *Batissa violacea*

*celebensis* Martens 1897 obtained, which are Bvc1, Bvc2, Bvc3, Bvc4, and Bvc5 isolates (Table 1). After being incubated for 24 hours, colonies of 5 bacterial isolates on NA media were observed macroscopically. Characteristics observed included colony shape, edge, elevation, color, and structure in the colony. Bvc1, Bvc3, and Bvc5 showed circular colonies with entire edges, or smooth. Bvc2 and Bvc4 showed irregular colonies with undulate edges, or wavy. All isolates showed low convex elevations that resembled water droplets, beige-colored with transparent inner structure.

In Table 1, we can see that the colony morphology of the five isolates showed nearly identical characteristics. In general, colony morphological characteristics could be used as a basis for identification at the genus level, but only a basis in differentiating between isolates[16]. Bacterial isolates can be said to be different if they have different colony characteristics[17]

Purified bacterial isolates were tested for their activities against MRSA pathogens using the paper disc method. The use of a single disc for each antibiotic with good standardization could determine whether bacteria are sensitive or resistant by comparing the standard resistance zones of the same drug [18]. The results of the qualitative inhibition ability test of bacterial isolates that were symbiotic with *Batissa violacea celebensis* Martens 1897 are shown in Table 2.

Based on the qualitative screening test, the five bacterial isolates exhibited different inhibitory ability against MRSA pathogenic bacteria. As shown in the results of the potential test in figure 2, there were two bacterial isolates, Bvc1, and Bvc3, that showed more potent inhibitory ability than the positive control. According to [19], different degrees of activity shown by each bacterial isolate may be caused by differences in secondary metabolites produced by each isolate. The positive control used was penicillin 1 g diluted with 9 ml ddH2O.

The inhibitory ability test conducted obtained two potential isolates as an anti-MRSA, namely Bvc1 and Bvc2. The activity of both isolates was more potent than control penicillin. The inhibitory ability of both isolates was in the sensitive category, in line with the Clinical and Laboratory Standards Institute (CLSI) stating that their inhibitory zones could be considered resistant (≤13 mm), intermediate (14-16 mm), sensitive (≥17mm). Penicillin was also resistant (≤13 mm), intermediate (14-16 mm), sensitive (≥17mm). The formation of inhibitory zone zones in BV1 and BV3 was a result of the antibacterial activities of resultant symbiont bacteria isolates. According to Yuliana (2014), symbiont bacteria can produce similar bioactive compounds as the host. In [7] study, *Batissa violacea celebensis* Martens 1897 contained bioactive compounds such as alkaloid, steroid, peptide, saponin, flavonoid, and phenol.

Studies on natural materials from the sea or rivers have also been carried out by several researchers, one of which was by [12] who used the symbiont bacteria in *Holothuriascabra* as anti-MRSA. The results of the study of 9 bacterial isolates found 6 isolates included in the sensitive category.

After the sensitive bacterial isolates have been identified, the process continued to the gram staining stage. Gram staining was performed to group the bacteria into 2, Gram-positive and Gram-negative bacteria. Based on Figure 2, it can be seen that the two isolates are Gram-positive with bacteria in the form of bacilli. Gram-positive bacteria have a cell wall structure with a thick peptidoglycan content, thus unaffected during alcohol decolorization. Gram-positive bacteria will retain the purple color of crystal violet that when observed under a microscope it will show a purple color[19]. So it can be concluded that both Bvc1 and Bvc3 isolates are classified as specific in inhibiting Gram-positive bacteria, this shows the potential for antibiotics that have specifications in inhibiting MRSA pathogenic target bacteria as shown by the two isolates to have high activity compared to penicillin in inhibiting the growth of MRSA bacteria.

## 5. Conclusion

Based on the results of the research, it is concluded that two bacterial isolates obtained from *Batissa violacea celebensis* Martens 1897 have the potential to be anti-MRSA.

**6. Conflict of Interest:**
The authors declare no conflict of interest.

**Acknowledgement**
Utmost gratitude to Mandala Waluya University and Hasanuddin University for facilitating the performance of this research.

## References

[1] Chen CJ & Huang YC. 2014. *New epidemiology of Staphylococcus aureus infection in Asia.*Clin Microbiol Infect. 20(7): 605-606.

[2] Cragg, G. M., and D. J Newman. 2013. *Natural products: A continuing source of novel drug leads.*Biochim. Biophys. Acta 1830:3670-3695.

[3]. Rasyid S A, Sugireng, Ridwan Adi Surya, Sanatang, Rosdarni, Wa Ode Rejeki Natalia. The antibacterial activity of Tembelekan leaf (Lantana camara L.) and Kopasanda leaf (Chromolaena odorata L.) extracts against Staphylococcus aureus. *Infectious Disease Reports 2020; 12(s1):8734 doi:10.4081/idr.2020.8734*

*[4]* Tokajian S. 2014. *New epidemiology of Staphylococcus aureus infections in the middle east.* Clinical Microbiology and Infection (CMI). 20(7): 624-628.

[5] Risky Yuliana Try, Agrijanti dan Nurul Inayati. 2019. *Uji Skrining Methicillin Staphylococcus Aureus (MRSA) Menggunakan Antibiotik Cefofitin 30 µg Pada Pasien Penderita Abses Gigi di Klinik BPJS Mataram*. Jurnal Analisis Medika Bio Sains. Vol.6 :98-104.

[6] Adriyani R dan Mahmudiono T. (2012). *Kadar LogamBerat Cadmium, Protein dan Organo leptik pada Daging Bivalvia dan Efektivitas Perendaman Larutan Asam Cuka.* Surabaya: Universitas Airlangga.

[7] Bahtiar, W. Nurgaya, dan L. Anadi. 2014. *Studi morfometrik dan meristic kerang pokea (Batissa violacea var. celebensis von Martens, 1897) yang tereksploitasi di Sungai Pohara Sulawesi Tenggara*. J. Biologi Tropis, 14(1):36-44.

[8] Grienke, U., Silke, J. dan Tasdemir, D. (2014) "Bioactive compounds from marine mussels and their effects on human health," *Food Chemistry*. Elsevier Ltd, 142, hal. 48–60. doi: 10.1016/j.foodchem.2013.07.027.

[9] Sernita, Adisya Yuning Pratiwi , Ahmad Lalo. 2016. *Uji Daya Hambat Ekstrak Kerang Pokea (Batissa violacea celebensis) Terhadap Bakteri Staphylococcus Aureus.* Warta Farmasi Vol.5 No.1, Issn 2089-712x

[10] Rochmawati I, Ibrahim M, Ambarwati R. 2015. *Aktivitas anti bakteri ekstrak kerang pisau (Solen sp.) dan kerang simping (Placuna placenta)*. Journal of Biology and Biology Education. 7(2): 128-135.

[11] Hasan T, Patong R, Wahab AW, Djide MN. 2014. *Isolasi dan Implementasi Protein Bioaktif Kepah (Atactodeastriata) sebagai Bahan Obat Anti bakteri.* Journal UIN Alauddin. 47-57.

[12] Rasyid S A, M Bintang, BP Priosoeryanto, RU Nurlila, RA Surya. 2018. *Analysis Chemical Compound of Pokea (Batissa violacea celebensis Martens 1897) The Oriign of Konawe Regency Southeast Sulawesi.* Indian Journal of Public Health Research & Development 9(6), 345-350.

[13] Sugireng dan Tiara Mayang Pratiwi Lio. 2019. *Skrining Bakteri Penghasil Senyawa Metabolit Anti-MRSA yang Bersimbiosis Dengan Holothuria Scabra Asal Perairan Tanjung Tiram.* Jurnal Biologi Makassar. Vol 5 (1):34-39.

[14] Kristiana,R., D. Ayuningrum, M.A. Asagabaldan, H. Nuryadi, A. Sabdono, O.K. Radjasa, A. Trianto. 2017. *Isolation and Partial Characterization of Bacteria Activity Associated with Gorgonian Euplexaura sp. Against Methicillin-Resistant Staphylococcus aureus (MRSA).* IOP Conf. Series: Earth and Environmental Science 55.

[15] Nismawati, Rizalinda Sjahril, Rosana Agus. 2018. *Deteksi Methicillin Resistant Staphylococcus Aureus (MRSA) Pada Pasien Rumah Sakit Universitas Hasanuddin dengan Metode Kultur*. Prosiding Seminar Nasional Megabiodiversitas Indonesia. Jurusan Biologi, Fakultas Saintek, UIN Alauddin. Makassar.

[16] Datta P, Gulati N, Singla N, Rani Vasdeva H, Bala K, Chander J, Gupta V. Evaluation of various methods for the detection of meticillin-resistant Staphylococcus aureus strains and susceptibility patterns. *J Med Microbiol. 2011 Nov;60(Pt 11):1613-1616. doi: 10.1099/jmm.0.032219-0. Epub 2011 Jul 21.* PMID: 21778267.

[17] Radjasa, O.K., Y.M. Vaske, G. Navarro, H.C. Vervoort, K.Tenney, R.G. Linington, P. Crews. 2011. *Highlights of marine invertebrate-derived biosynthetic products: Their biomedical potential and possible production by microbial associants*. Bioorganic & Medicinal Chemistry 19: 6658–6674

[18] Fatimah S, Nadifah F, Burhandin I. 2016. *Uji daya hambat ekstrak etanol kubis (Brassica oleracea var. capitata f. alba) terhadap bakteri Staphylococcus aureus secara in vitro*. Biogenesis. 4(2): 102-106.

[19] Mans, D.R.A. 2016. *Exploring the global animal biodiversity in the search for new drugs marine invertebrates*. J Transl Sci. 2(3): 170-179. doi: 10.15761/ JTS. 1000136

[20] Erwin, P. M., M. C. Pineda, N.Webster, X.Truon, S. López-Legentil. 2014. *Down under the tunic: bacterial biodiversity hotspots and widespread ammoniaoxidizing archaea in coral reef ascidians*. ISME J. 8:575-588.