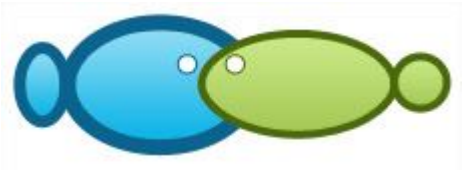

Enterococcus gallinarum, a new antibiotic-producing bacterium against fish pathogenic bacteria, isolated from mangrove mud snake (*Cerberus rynchops*)

Dessy Yoswaty, Irwan Effendi, Muhammad R. Titani

Faculty of Fisheries and Marine Sciences, University of Riau, 28295 Pekanbaru, Indonesia. Corresponding author: I. Effendi, helpingirwan@gmail.com

Abstract. The study aimed to identify antibiotic-producing bacteria isolated from mangrove mud snake (*Cerberus rynchops*) gut against fish pathogenic bacteria *Aeromonas hydrophila*, *Aeromonas salmonicida* and *Edwardsiella ictaluri*. Samples of 0.01-0.1 mL lower gut were diluted into a sterile physiological solution, and plated on nutrient agar (NA) and tryptic soy agar (TSA) media. Well grown colonies were selected and screened for their ability to produce antibiotics against pathogenic bacteria by the streaking method on the media. The test was preceded by paper disc diffusion method on the same media. A number of 18 potential antibiotic-producing isolates were then identified through a series of phenotypic and genotypic tests. An isolate, considered as the most potential one (U1c), was selected and identified molecularly. The results of DNA analysis using the 16S rRNA PCR method and BLAST analysis revealed that the U1c isolate was identified as *Enterococcus gallinarum*.

Key Words: *C. rynchops*, antibiotic-producing bacteria, *A. hydrophila, A. salmonicida, E. ictaluri, E. gallinarum*.

Introduction. Fish disease is still a serious problem in the aquaculture industry. *Aeromonas hydrophila, Aeromonas salmonicida*, and *Edwardsiella ictaluri* are examples of common fish pathogens (Austin & Austin 2012; Aisiah et al 2019). Today, antibiotic is still one of the most important commercially exploited secondary metabolites produced by bacteria and fungi (Langaoen et al 2018). The use of bacteria from the sea as producers of inhibitory substances against pathogenic bacteria in aqua systems has become more common as an efficacious alternative to ensure the organisms health, and minimizing the use of antimicrobials (Valenzuela et al 2009; Concha-Meyer et al 2011).

Soil, water and weathered or rot organic matter are common sources of antibiotic-producing microorganisms. Nowaday, some extreme microhabitats from the above general habitats have started to become the source of these microorganisms (Clardy et al 2009; Bizuye et al 2013; Begum et al 2017). Environmental sources have been crucial in the discovery of important bioactive compounds producing bacteria, including antibiotics, immunosuppressive drugs, anticancer drugs, and other biologically active chemicals. In recent years, much of the attention was focused on unexplored or more extreme environment habitats such as deep-sea, desert, cryo, and volcanic environments for the isolation of potential bacteria such as *Streptomyces* species (Pathom-Aree et al 2006; Romanenko et al 2013; Li et al 2014; Skropeta & Wei 2014). Certain parts of alive plants and animals, for instance, lower digestive tract of mangrove mud snake (*Cerberus rhynchops*) is an example of such extreme or unexploited microhabitat.

Mangrove mud snake is one of biota that inhabits the mangrove ecosystem. The snake is relatively small in size, less venomous, inhabiting holes in mud in the mangrove forest, and native to coastal waters of South and Southeast Asia. This is a saltwater-tolerant species found in India (including Andaman and Nicobar Islands), Sri Lanka, North Western Pakistan (Khyber Pakhtunkhwa), Bangladesh, Myanmar, the Philippines, Indonesia and Thailand (Karns et al 2000; Murphy et al 2012). As an aquatic

and nocturnal snake, it feeds mainly on fish, worm and other invertrates (Karns et al 2000; Murphy et al 2012).

This study aimed to isolate and identify the antibiotic-producing bacteria from extreme microhabitats, namely mangrove mud snake lower gut against the fish pathogenic bacteria.

Material and Method

Collecting sample. A number of 6 snakes were caught by using a fishing landing net and followed bare hand catch from mangrove ecosystem Sungai Kayuara, Siak, Riau, Indonesia, during the months of July, August and September 2019. These animals were then kept in an iced box and taken to the laboratory. The snakes were then killed by hitting the nape of the neck and dissected. Samples of 0.01-0.1 mL lower gut content were taken from the snakes. The samples were diluted into a sterile physiological solution, and plated on nutrient agar (NA; Oxoid) and tryptic soy agar (TSA; Oxoid) media. Well grown colonies were selected and tested for their ability to produce antibiotics against fish pathogenic bacteria, *A. hydrophila, A. salmonicida*, and *E. ictaluri* (isolated fom some diseased fish in Sumatera, Indonesia). Streaking method on muller hilton agar (MHA;Oxoid) media was applied (Effendi & Austin 1991). The test was proceeded to paper disc diffusion method (Austin et al 1993) on MHA medium. Positive results was indicated by a clear zone around the disc paper.

Phenotypical identification. A number of 18 potential antibiotic-producing isolates were then identified through a series of phenotypic (morphology and physiology) and genotypic tests. Morphologically the color, shape, size, margin and elevation of the colonies were observed, and the cells were Gram stained. Physiological tests included catalase production, sulfide production (H_2S), sugar use, motility, citrate production, and methyl red test. All of these tests were carried out based on Lapage (1976), Odds (1981), MacFaddin (2000), and Hemraj et al (2013).

Genotypical identification. An isolate (U1c), considered as the most potential one, was selected and identified molecularly. DNA of the bacteria were extracted using DNA easy mini column and amplified by using Kappa PCR kit with 16S rDNA 1A and 16S rDNA2A primers. The PCR results were analyzed on a 0.5% agarose gel in Tris-acetat-EDTA (TAE) and electrophoresis buffer (4 mM Tris-acetate and 1 mM disodium EDTA at pH 8.0). The electrophoresis process was carried out at 60 mA (100-120 v) for 30-45 minutes and read by UV transilluminator (Singh et al 1973).

The PCR result from bacterial isolate was sent to PT. Genetics Science, Jakarta for DNA sequencing in the 16S rRNA region. The nucleotide sequences were then analysed by comparing it to sequence databases by BLAST (basic local alignment search tool). Furthermore, the relationship in phylogenetic tree was analyzed by using the maximum parsimony method and neighbor joining with 1000 bootstrap resampling.

Result and Dicussion. A number of 300 well grown bacterial colonies were examined for their potential against the fish pathogens. Through streaking method, as many as 18 bacterial isolates, expected to be potential antibiotics producing bacteria, were isolated in initial screening. These isolates were further purified and identified by observing colony morphology; diameter, color, shape, edge and surface of each colony (Table 1).

Identification of selected bacterial isolates was then further pursued by serial biochemical tests, including Gram staining, motility, catalase, indole, citrate, sulfide and methyl red tests, and sugar usage test (Table 2).

Confirmation of the ability of bacterial isolates to produce antibiotics to the fish pathogenic bacteria was further examined through the paper disc diffusion method. The existence of inhibition and wide clear zone around the disc paper is considered as an indication of producing antibiotics. The diameter of the clear zone was measured and averaged. This study showed that these isolates have different abilities in inhibiting pathogenic bacteria *A. hydrophylla, A. salmonicida* and *E. ictaluri*. Generally the isolates

obtained did not show good inhibition. However, one isolate (U1c) exhibited a high inhibitory properties (Table 3).

Table 1

Morphology of potential bacterial colonies against pathogenic bacteria *A. hydrophylla, A. salmonicida* and *E. ictaluri*

No.	*Isolate code*	*Diameter (cm)*	*Colony colour*	*Shape*	*Colony edge shape*	*Colony elevation*
1	U1a	1.2	White yellowish	Round	Irregular	Raised
2	No	2.3	White yellowish	Round	Smooth	Raised
3	U1c	2.2	Milky white	Round	Smooth	Flat
4	U1d	2	Milky white	Round	Wavy	Flat
5	U1e	2.5	Milky white	Punctiform	Smooth	Unbonate
6	U1f	2.1	Milky white	Punctiform	Smooth	Raised
7	U2a	1.5	Milky white	Round	Irregular	Flat
8	U2b	1.7	White yellowish	Round	Smooth	Raised
9	U2c	1.9	White yellowish	Irregular	Wavy	Unbonate
10	U2d	2.5	White yellowish	Round	Irregular	Unbonate
11	U2e	2.5	White yellowish	Round	Smooth	Flat
12	U2f	1.6	Milky white	Round	Irregular	Flat
13	U2g	2.4	Milky white	Punctiform	Irregular	Unbonate
14	U3a	2	Milky white	Irregular	Smooth	Unbonate
15	U3b	2.4	Milky white	Round	Smooth	Raised
16	U3c	1.4	Milky white	Round	Wavy	Raised
17	U3d	1.8	Milky white	Irregular	Wavy	Raised
18	U3e	1.1	White yellowish	Round	Smooth	Flat

Table 2

Biochemical tests of potential bacterial isolates against pathogenic bacteria *A. hydrophylla, A. salmonicida* and *E. ictaluri*

No.	*Isolate code*	*Gram staining*	*Catalase*	*Citrate*	*Sulfide*	*Motility*	*Indol*	*MR*	*Glucose*	*Lactose*	*Sucrose*
1	U1a	+	+	-	-	-	+	-	-	-	-
2	U1b	+	+	-	-	+	-	-	+	+	+
3	U1c	+	+	+	-	-	-	+	+	+	+
4	U1d	+	+	+	-	+	-	-	-	-	-
5	U1e	-	+	+	+	+	-	-	+	+	+
6	U1f	-	+	-	+	+	+	+	-	+	+
7	U2a	+	+	-	+	+	-	-	-	+	+
8	U2b	-	+	+	+	+	-	-	-	+	+
9	U2c	+	+	+	+	-	-	-	+	+	+
10	U2d	-	-	+	+	+	+	-	-	-	-
11	U2e	+	-	-	+	+	-	+	-	-	-
12	U2f	+	+	+	+	-	-	+	+	-	-
13	U2g	-	-	+	-	-	+	+	+	-	-
14	U3a	+	+	-	+	+	+	-	+	-	-
15	U3b	+	-	-	-	+	+	-	+	-	-
16	U3c	+	-	+	-	+	-	-	+	+-	+
17	U3d	-	-	+	-	+	-	+	+	+	+
18	U3e	-	-	+	+	+	-	+	+	-	-

Table 3

Results of confirmation test of antibiotic production and inhibitory zone (mm) of isolates against pathogenic bacteria *A. hydrophylla, A. salmonicida* and *E. ictaluri*

No.	*Isolates code*	*A. hydrophila*	*A. salmonicida*	*E. ictaluri*
1	U1a	2.3	3.6	2.0
2	U1b	5.0	4.5	1.0
3	U1c	19.6	16.6	13.6
4	U1d	2.0	3.3	1.2
5	U1e	5.3	0.0	5.0
6	U1f	0.0	3.2	5.2
7	U2a	1.8	4.3	2.8
8	U2b	6.0	5.8	7.5
9	U2c	9.7	5.5	4.8
10	U2d	6.8	8.2	0.0
11	U2e	0.0	0.0	0.0
12	U2f	1.8	3.3	0.0
13	U2g	0.0	0.0	0.0
14	U3a	2.3	2.1	1.8
15	U3b	2.5	4.1	1.5
16	U3c	0.6	0.8	4.1
17	U3d	2.8	0.0	1.0
18	U3e	2.9	2.2	0.0

The total extracted DNA of U1c isolate was then electrophoresed using 0.5% agarose (Figure 1). This total DNA was then replicated through the PCR process until it reached ±1500 bp (base pair). The DNA of this PCR product was then electrophoresed using 1% agarose gel (Figure 2).

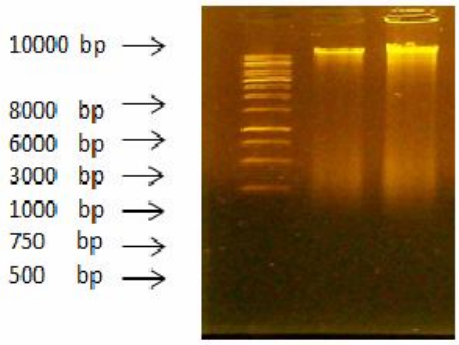

Figure 1. Electrophoresis of total DNA extract in 0.5% agarose gel.

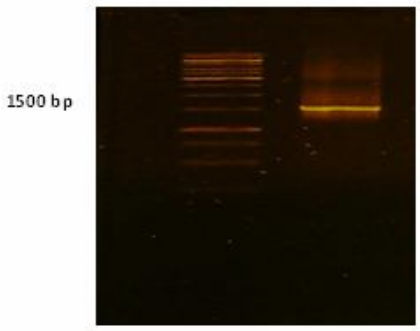

Figure 2. Electrophoresis of PCR products in 1% agarose gel.

The total DNA electrophoresis (Figure 1) clearly visiblelysed that DNA bands with a large size above 10,000 bp, whereas in Figure 2 the large band size of the resulting PCR product was 1500 bp. The thickness of the band indicates that the isolate has sufficient DNA. This size corresponds to the expected size of the 16S rRNA genes of 1500-1600 bp. BLAST analysis is a species name search system for an organism based on the level of DNA homology resulting from sequencing of isolates compared with a data base that already exists in Gen Bank through the website of http://www.ncbi.nlm.nih.gov. The results of identification of U1c isolates based on BLAST are presented in Table 4.

From Table 4 it is noted that the U1c bacterial isolate has a homology level of 97.22% against *Enterococcus gallinarum* strain 166-a white bacteria. Judging from the level of homology (97.22%) U1c isolates can represent at the same species level (Figure 3). Hung & Weng (2016) stated that if homology has a percentage close to 100% or above 97% it can be confirmed as the same species. Conversely, if the homology is lower than 97%, it is likely that the isolate is a new species.

Table 4

The BLAST analysis of isolate U1c

Species	*Origin*	*Strain*	*Acces code*	*References*	*Homology*
Enterococcus gallinarum	Wolf feces	46W	MK929299.1	Dec et al (2020)	97.22%
Enterococcus gallinarum	Human wound	16S	LN829114.2	Loong et al (2016)	97.22%
Enterococcus gallinarum	Wolf feces	166-a white	MN208191.1	Deng (2019)	97.22%
Bacterium	8 weeks pig feces	NLAE-zl-P779	JQ607614.1	Ziemer (2014)	97.36%
Bacterium	8 weeks pig feces	NLAE-zl-P715	JQ607501.1	Ziemer (2014)	97.36%
Uncultured organism	Gastrointestinal specimen	ELU0043	HQ759124.1	Li et al (2014)	97.22%
Uncultured *Enterococcus* sp.	Milk	Clone 49	KM030265.1	Hou et al (2015)	97.22%
Uncultured bacterium	Sea water	Clone W2-79	MF498221.1	Kolm et al (2018)	97.22%

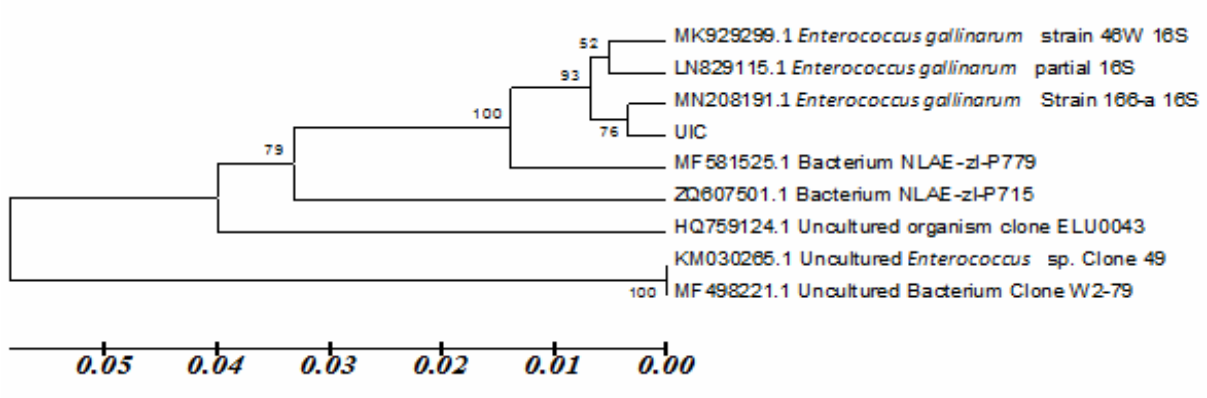

Figure 3. Philogenetic tree of isolate U1c, *Enterococcus gallinarum*.

In this study, based on its morphological, biochemical and 16S rDNA properties, the results of identification are consistent with some previous researchers report. U1c isolate is Gram positive and non-motile. The colony shape is round, milky white in color, wavy edge, and flat elevation. Catalase positive, indole negative, and MR test negative. Eshaghi et al (2015) reported that *E. gallinarum* as Gram-positive bacterium, negative catalase and positive motility. *E. gallinarum* is a facultative anaerobic organism (Fisher & Phillips 2009).

The presence of *E. gallinarum* bacterium in mangrove ecosystems, especially in snake intestine is considered to be normal, since this bacterium is usually found in the intestines of mammals, and appears in feces, soil and water as fecal contamination. According to Monticelli et al (2018). *E. gallinarum* is relatively rare in humans and poultry. However, the bacterium can also be found in human food, for example in fish, beef, a combination of cheese and meat, minced beef and minced pork. *Enterococcus* spp. can be easily isolated from soil, water, plants, humans, animals, fish and crustaceans intestines and bivalve mollusks (Martines & de Oliveira 2010). *Enterococcus* is known to be very tolerant to various environmental conditions. This allows them to grow over a wide range variety of microhabitats. The temperature range is relatively extreme (10-45°C) but the optimal growth temperature of *Enterococcus* is 37°C, growth can occur in the pH range of 4.4-10.6 (Fisher & Phillips 2009). Ogier & Serror (2008) and Hammad et al (2014) also reported that the bacterium survives in environments with salt concentrations up to 6.5%, pH between 4.0 and 9.6 and temperatures between 10 and 45°C.

The antibiotic-producing bacteria isolated from marine ecosystem have been reported by some researchers. The bacteria are widely distributed in the nature, where they play an important role in regulating the microbial population of soil, water, sewage, and compost. Effendi et al (2020) reported that *Bacillus amyloliquefaciens, Bacillus cereus, Enterobacter hormaechei,* and *Klebsiella pneumonia* produced inhibitory compound against fish pathogens *A. hydrophila, Vibrio alginolyticus, Edwarsiella tarda* and *Escherichia coli*. *B. amyloliquefaciens* B94 strain produces iturin A used as biocontrol to suppress the disturbing fungi of *Rhizoctonia solani* plants (Yu et al 2002), *B. amyloliquefaciens* GA1 was reported as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens (Arguelles-Arias et al 2009), and *B. cereus* strain SU12 was indicated as a high capacity enzyme producer of proteinase (Umayaparvathi et al 2013), an inhibiting enzyme to some other microbes. *Enterobacter hormaechei* is one of the species in Enterobacter group producing an extended-spectrum beta-lactamase (ESBL). This enzyme is inhibiting and even killing other bacteria (Said et al 2015).

The finding of this work is insteresting since so far there is only one report informing that *E. gallinarum* is antibiotic producing bacterium. Jennes et al (2000) notified *E. gallinarum* strain 012, isolated from the duodenum of ostrich, produced enterocin 012 which is active against *E. faecalis, Lactobacillus acidophilus, Lactobacillus sakei, Listeria innocua, Propionibacterium acidipropionici, Propionibacterium* sp., *Clostridium perfringens, Pseudomonas aeruginosa* and *Salmonella typhimurium*. However, bioactive compound inhibiting effect from other species of *Enterobacter* genus has been reported by some researchers. *E. faecium* has been reported to inhibit biofilm formation by cariogenic bacteria *Streptococci* (Kumada et al 2009; Suzuki et al 2011). The inhibitory activity presented by *E. faecalis* isolates is promising regarding exploitation of its biotechnological potential, especially when considering microorganisms with probiotic potential for aquaculture (De Souza Carneiro et al 2015).

The antagonism of *Enterococcus* with other bacteria has been studied also by some other researchers. The genus has been indicated in the selection of potential probiotic bacteria (Concha-Meyer et al 2011). De Souza Carneiro et al (2015) reported that 18 strains of *Enterococcus* isolated from marine ecosystem (sea water, oysters *Crassostrea rhizophorae* and sururu *Mytella guyanensis*) inhibited *Lactobacillus lactis* (5 isolates), *L. inoccua* (1 isolate), *L. monocytogenes* (1 isolate), *Micrococcus luteus* (4 isolates) *Enterococcus faecalis* (5 isolates), and *Staphylococcus aureus* (2 isolates).

Microorganisms may produce several inhibitory substances, such as metabolism sub products: lactic acid, diacetyl, hydrogen peroxide, lithic agents, exotoxins and bacteriocins (Schillinger & Lücke 1989). The study on *Enterococcus* bacteriocins producer has gained interest since it can be used as biocontrol to pathogenic bacteria such as *L. monocytogenes*. Bacteriocin production by some bacterial isolates from seafood was reported by Valenzuela et al (2010) and Pinto et al (2009). Bacteriacin ST15, produced by *Enterococcus mundtii*, has also been reported (De Kwaadsteniet et al 2005). *Enterococcus* spp. can produce some proteases that help them to survive in the medium where they live and produced only when necessary (Park et al 2007).

Conclusions. This research succeeded in isolating antibiotic-producing bacteria to fish pathogenic bacteria of *A. hydrophylla, A.salmonicida* and *E. ictaluri*. The bacterium was isolated from the gut of mangrove mud snakes. Phenotive and genotive tests identified the isolate as *Enterococcus gallinarum*. It is hoped that this finding will contribute to solve the problem in fish diseases, at least caused by the above pathogenic bacteria.

References

Aisiah S., Prajitno A., Maftuch, Yuniarti A., 2019 The potential of bangkal leaf (*Nauclea subdita* [Korth.] Steud.) extract as antibacterial in catfish *Pangasius hypophthalmus* culture. AACL Bioflux 12(6):2093-2102.

Arguelles-Arias A., Ongena M., Halimi B., Lara Y., Brans A., Joris B., Fickers P., 2009 *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. Microbial Cell Factories 8:63.

Austin B., Austin D. A., 2012 Bacterial fish pathogens - disease of farmed and wild fish. 5th Edition. Springer, Netherlands, 654 pp.

Austin B., Gibb A., Effendi I., 1993 Developments in the biology of the fish pathogen *Aeromonas salmonicida.* Medical Microbiology Letters 2(6):329-334.

Begum K., Mannan S. J., Rahman M. M., Mitchel-Antoine A., Opoku R., Burgos N., Shepherd J., Ahmed I., Nur-E-Kamal A., 2017 Identification of antibiotic producing bacteria from soil samples of Dhaka, Bangladesh. Journal of Microbiology and Experimentation 4(6):00134.

Bizuye A., Moges F., Andualem B., 2013 Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pacific Journal of Tropical Disease 3(5):375-381.

Clardy J., Fischbach M., Currie C., 2009 The natural history of antibiotics. Current Biology 19(11):437-441.

Concha-Meyer A., Schôbitz R., Brito C., Fuentes R., 2011 Lactic acid bacteria in an alginate film inhibit *Listeria monocytogenes* growth on smoked salmon. Food Control 22(3-4):485-489.

De Kwaadsteniet M., Todorov S. D., Knoetze H., Dicks L. M. T., 2005 Characterization of a 3944 Da bacteriocin, produced by *Enterococcus mundtii* ST15, with activity against Gram-positive and Gram-negative bacteria. International Journal of Food Microbiology 105(3):433-444.

De Souza Carneiro C., Evangelista-Barreto N. S., da Silveira-Oliveira C. S., Silva I. P., de Oliveira T. A. S., Saraiva M. A. F., 2015 Antagonistic activity, antimicrobial susceptibility and potential virulence factors of *Enterococcus faecalis*. Journal of Life Sciences 9:318-326.

Dec M., Stepien-Pysniak D., Gnat S., et al, 2020 Antibiotic susceptibility and virulence genes in *Enterococcus* isolates from wild mammals living in Tuscany, Italy. Microbial Drug Resistance 26(5):505-519.

Deng H. Y., 2019 *Enterococcus gallinarum* strain 166-a white 16S ribosomal RNA gene, partial sequence. Report. Institute of Microbiological Lab, Lanzhou CDC, Center for Disease Control and Prevention in Lanzhou, 999# East of Nanbinhe Street, Chengguan District, Lanzhou, Gansu 730030, China.

Effendi I., Austin B., 1991 Survival of the fish pathogen *Aeromonas salmonicida* in seawater. FEMS Microbiology Letters 84(1):103-106.

Effendi I., Tanjung A., Sari D. M., 2020 Isolation of antibiotic-producing bacteria from extreme microhabitates in mangrove ecosystem. Journal of Physics: Conference Series 1655:011002.

Eshaghi A., Shahinas D., Li A., Karyawasam R., 2015 Characterization of an *Enterococcus gallinarum* isolate carrying a dual vanA and vanB cassette. Journal of Clininical Microbiology 53(7):2225-2229.

Fisher K., Phillips C., 2009 The ecology, epidemiology and virulence of *Enterococcus*. Microbiology 155(6):1749–1757.

Hammad A. M., Shimamoto T., Shimamoto T., 2014 Genetic characterization of antibiotic resistance and virulence factors in *Enterococcus spp*. from Japanese retail ready-to-eat raw fish. Food Microbiology 38:62-66.

Hemraj V., Diksha S., Avneet G., 2013 A review on commonly used biochemical test for bacteria. Innovare Journal of Life Sciences 1(1):1-7.

Hou Q., Xu H., Zheng Y., Xi X., Kwok L., Sun Z., Zhang H., Zhang W., 2015 Evaluation of bacterial contamination in raw milk, ultra-high temperature milk and infant formula using single molecule, real-time sequencing technology. J. Dairy Sci. 98:8464–8472. http://dx.doi.org/10.3168/jds.2015-9886

Hung J. H., Weng Z. P., 2016 Sequence alignment and homology search with BLAST and ClustalW. Cold Spring Harbor Protocols 2016(11):pdb.prot093088.

Jennes W., Dicks L. M., Verwoerd D. J., 2000 Enterocin 012, a bacteriocin produced by *Enterococcus gallinarum* isolated from the intestinal tract of ostrich. Journal of Applied Microbiology 88(2):349-357.
Karns D. R., O'Bannon A., Voris H. K., Weigt L. A., 2000 Biogeographical implications of mitochondrial DNA variation in the Bockadam snake (*Cerberus rynchops*, Serpentes, Homalopsinae) in Southeast Asia. Journal of Biogeography 27:391–402.
Kolm H. E., Gomes K. V., Ishii F. G., Martins C. C., 2018 An integrated appraisement of multiple faecal indicator bacteria and sterols in the detection of sewage contamination in subtropical tidal creeks. International Journal of Hygiene and Environmental Health 221(7):1032-1039.
Kumada M., Motegi M., Nakao R., Yonezawa H., Yamamura H., Tagami J., Senpuku H., 2009 Inhibiting effects of *Enterococcus faecium* non-biofilm strain on *Streptococcus mutans* biofilm formation. Journal of Microbiology, Immunology and Infection 42(3):188-196.
Langaoen A. F., Manzano V. J. V., Requilman E. M. R., Tabardillo J. M., Maningas M. B. B., Calugay R. J., 2018 Antibiotic-resistant bioluminescent vibrios from Philippine aquacultured *Chanos chanos* and *Oreochromis niloticus*. AACL Bioflux 11(2):505-515.
Lapage S. P., 1976 Biochemical tests for identification of medical bacteria. Journal of Clincal Pathology 29(10):958-969.
Li P., Wu D., Liu K., Suolang S., He T., Liu X., Wu C., Wang Y., Lin D., 2014 Investigation of antimicrobial resistance in *Escherichia coli* and enterococci isolated from Tibetan pigs. PLoS ONE 9(4):e95623.
Loong S. K., Che Mat Seri N. A. A., Mahfodz N. H., Ahmad Nasrah S. N., Teoh B. T., AbuBakar S., 2016 Emergence of *Enterococcus gallinarum* carrying vanA gene cluster displaying atypical phenotypes. Tropical Biomedicine 33(4):837-841.
MacFaddin J. F., 2000 Biochemical tests for identification of medical bacteria. 3rd Edition, Jones & Bartlett Learning, Massachusetts, USA, 912 pp.
Martines D. I., de Oliveira A. J. F. C., 2010 Faecal bacteria in *Perna perna* (Linnaeus, 1758) (Mollusca: bivalvia) for biomonitoring coastal waters and seafood quality. Brazilian Journal of Oceanography 58(3):29-35.
Monticelli J., Knezevich A., Luzzati R., 2018 Clinical management of non-*faecium* non-*faecalis* vancomycin-resistant enterococci infection. Focus on *Enterococcus gallinarum* and *Enterococcus casseliflavus/flavescens.* Jurnal of Infection and Chemotherapy 24(4):237-246.
Murphy J. C., Voris H. K., Karns D. R., 2012 The dog-faced water snakes, a revision of the genus *Cerberus* Cuvier, (Squamata, Serpentes, Homalopsidae), with the description of a new species. Zootaxa 3484:1-34.
Odds F., 1981 Biochemical tests for identification of medical bacteria. Journal of Clinical Pathology 34(5):572.
Ogier J. C., Serror P., 2008 Safety assessment of dairy microorganisms: the *Enterococcus* genus. International Journal Food Microbiology 126(3):291-301.
Park S. Y., Kim K. M., Lee J. H., Seo S. J., Lee I. H., 2007 Extracellular gelatinase of *Enterococcus faecalis* destroys a defense system in insect hemolymph and human serum. Infection and Immunity 74(4):1861-1869.
Pathom-Aree W., Stach J. E. M., Ward A. C., Horikoshi K., Bull A. T., Goodfellow M., 2006 Diversity of actinomycetes isolated from challenger deep sediment (10,898 m) from the Mariana trench. Extremophiles 10(3):181-189.
Pinto A. L., Fernandes M., Pinto C., Albano H., Castilho F., Teixeira P., Gibbs P. A., 2009 Characterization of anti-*Listeria* bacteriocins isolated from shellfish: potential antimicrobials to control non-fermented seafood. International Journal of Food Microbiology 129(1):50-58.
Romanenko L. A., Tanaka N., Kalinovskaya N. I., Mikhailov V. V., 2013 Antimicrobial potential of deep surface sediment associated bacteria from the Sea of Japan. World Journal of Microbiology and Biotechnology 29(7):1169-1177.

Said L. B. A., Jouini N., Klibi R., Dziri R., Alonso A., Boudabous A., Slama K. B., Torres C., 2015 Detection of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. International Journal of Food Microbiology 203:86-92.
Schillinger U., Lücke F. K., 1989 Antibacterial activity of *Lactobacillus sake* isolated from meat. Applied and Environmental Microbiology 55(8):1901-1906.
Skropeta D., Wei L. Q., 2014 Recent advances in deep-sea natural products. Natural Product Reports 31(8):999-1025.
Singh R. S., Jain S. K., Qualset C. O., 1973 Protein electrophoresis as an aid to oat variety identification. Euphytica 22:98-105.
Suzuki N., Yoneda M., Hatano Y., Iwamoto T., Masuo Y., Hirofuji T., 2011 *Enterococcus faecium* WB2000 inhibits biofilm formation by oral cariogenic streptococci. International Journal of Dentistry 2011:834151.
Umayaparvathi S., Meenakshi S., Arumugam M., Balasubramanian T., 2013 Purification and characterization of protease from *Bacillus cereus* SU12 isolated from oyster *Saccostrea cucullata*. African Journal of Biotechnology 12(40):5897-5908.
Valenzuela A. S., Omar N. B., Abriouel H., et al, 2009 Virulence factors, antibiotic resistance, and bacteriocins in enterococci from artisan foods of animal orgin. Food Control 20:381-385.
Valenzuela A. S., Benomar N., Abriouel H., Cañamero M. M., Gálvez A., 2010 Isolation and indentification of *Enterococcus faecium* from seafoods: antimicrobial resistance and production of bacteriocin-like substances. Food Microbiology 27(7):955-961.
Yu G. Y., Sinclair J. B., Hartman G. L., Bertagnolli B. L., 2002 Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani.* Soil Biology and Biochemistry 34(7):955-963.
Ziemer C. J., 2014 Newly cultured bacteria with broad diversity isolated from eight-week continuous culture enrichments of cow feces on complex polysaccharides. Applied Environmental Microbiology 80(2):574-585.

Received: 20 July 2020. Accepted: 30 October 2020. Published online: 22 December 2020.
Authors:
Dessy Yoswaty, Faculty of Fisheries and Marine Sciences, University of Riau, 28295 Pekanbaru, Indonesia, e-mail: dyoswaty@yahoo.com
Irwan Effendi, Faculty of Fisheries and Marine Sciences, University of Riau, 28295 Pekanbaru, Indonesia, e-mail:helpingirwan@gmail.com
Muhammad R. Titani, Faculty of Fisheries and Marine Sciences, University of Riau, 28295 Pekanbaru, Indonesia, e-mail: aik_irwan@yahoo.co.id

How to cite this article:
Yoswaty D., Effendi I., Titani M. R., 2020 *Enterococcus gallinarum*, a new antibiotic-producing bacterium against fish pathogenic bacteria, isolated from mangrove mud snake (*Cerberus rynchops*). AACL Bioflux 13(6):3769-3777.