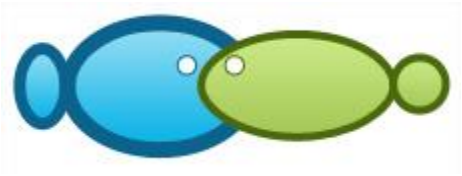

Activity of an antibacterial compound produced by *Bacillus* sp. strain JS4 (MT102913.1)

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Abstract. Pathogenic bacteria are types of bacteria that often cause negative effects on fish and other organisms. *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, and *Vibrio alginolyticus* bacteria often infect farmed fish, causing death. The use of antibiotics to treat bacterial diseases can result in antibiotic residues in aquatic products and bacterial resistance. The heterotrophic microscopic organisms separates were gathered from ocean waters in Sungai Pakning Bengkalis Rule Riau Territory Indonesia. The execution season of this study was directed in May-November 2020. An antibacterial compound from *Bacillus* sp. strain JS4 can be used as an alternative to solve this problem. 12 liters of *Bacillus* sp. strain JS4 bacterium was cultured for 7 days on NB media, then extracted with ethyl acetate. The concentrate was disconnected utilizing streak chromatography. The concentrate was identified utilizing FT-IR and the compound was portrayed utilizing 1H NMR (Nuclear Magnetic Resonance) at 500 MHz recurrence and 13C NMR at 125 MHz. The compound test was finished by utilizing the agar dissemination strategy. The consequence of range examination NMR and Comfortable confirmed that compound JS4-E-01 was N-[2-(3-hydroxypropanoyl)ethenyl]3-hydroxypropanamide. The restraint ability test JS4-E-01 on *A. hydrophila* (10.5 mm), *P. aeruginosa* (12.3 mm), and *V. alginolyticus* (13,3 mm). All in all, *Bacillus* sp. strain JS04 bacterium can deliver N-[2-(3-hydroxypropanoyl)ethenyl]3-hydroxypropanamide compound. This compound had the option to restrain the development of *A. hydrophila*, *P. aeruginosa* and *V. alginolyticus* microbe microscopic organisms.
Key Words: aquaculture, bacteria, fish pathogens.

Introduction. Pathogenic bacteria are types of bacteria that often cause negative effects on fish and other organisms. *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Vibrio alginolyticus* bacteria often infect farmed fish (Chen et al 2019; Zhang et al 2016; Stratev & Odeyemi 2016). As a result of this infection, fish die causing huge losses (Cai et al 2018; Liu et al 2017). *Aeromonas* spp. Bacteria is found in fresh water, estuarine and marine waters, and is an etiological agent of fish disease (Chaix et al 2017). Infection with *A. hydrophila* can cause degenerative changes, necrosis and swelling on the internal organs of the fish (Abdelhamed et al 2017). *A. hydrophila* bacteria produce virulence factors, such as hemolysins, aerolysins, adhesins, enterotoxins, phospholipases, and lipases (Stratev & Odeyemi 2016). *A. hydrophila* can quickly multiply through the fish's circulatory system and cause death within 8 to 24 hours (Zhang et al 2016).

Pseudomonas aeruginosa is a gram-negative bacterium, which is opportunistic in aquaculture systems that can cause liver damage to fish (Souza et al 2019). *P. aeruginosa* also can live in various environmental conditions, this characteristic allowing *P. aeruginosa* bacteria to become infectious agents (Lee & Zhang 2015). *P. aeruginosa* can produce various poisons with hydrolytic activity (Weiler et al 2022). In the process of infection, *P. aeruginosa* depends on virulence factors, which allows it to move, attach to host cells, avoid the immune response, and migrate within the organism (Gellatly & Hancock 2013; Hilliam et al 2020).

Vibrio alginolyticus is a pathogen that causes vibriosis in fish species. *V. alginolyticus* bacteria has been associated with several diseases in fish and shrimp

aquaculture and has caused huge economic losses (Liu et al 2017). *Vibrio* sp. bacteria causes disease in tiger prawns, with clinical symptoms of abnormal swimming and decreased appetite (Feliatra et al 2018). Epizootic disease begins with clinical signs followed by an acute death. The infection causes bleeding in the gastric mucosa. Histologically, the gastric mucosa undergoes necrosis in *Rachycentron canadum* fish (Rameshkumar et al 2017).

Antibiotics are widely used in aquaculture. The use of antibiotics to treat bacterial diseases can result in antibiotic residues left behind in aquatic products and bacterial resistance (Chen et al 2020; Rodriguez-Mozaz et al 2020). So, it is necessary to look for natural antibacterial compounds derived from microorganisms. Many marine microorganisms produce compounds that have biological activity (Gozari et al 2021). Several species of *Bacillus* bacteria can produce antibacterial compounds such as *Bacillus pumilus*. It produces compounds amicoumacin A, amicoumacin B, phosphoamicoumacin A and phosphoamicoumacin B (Zidour et al 2017). *Bacillus amyloliquefaciens* produces 4 types of compounds (Chakraborty et al 2018). Bacillus subtilis MTCC 10403 produces 4 types of compounds (Chakraborty et al 2017). *Bacillus* sp. produce pumilacidin compounds (Xiu et al 2017). This research is a continuation of previous research (Setiaji et al 2020) which aims to characterize the JS4-E-01 compound produced by the *Bacillus* sp. strain JS4 bacteria, which has the potential to inhibit the growth of pathogenic bacteria.

Material and Method

Extraction and isolation. This study was conducted in May-November 2020. The heterotrophic microscopic organisms were gathered from ocean waters in Sungai Pakning Bengkalis Regime Riau Area Indonesia (North scope 01° 21'36,8" and East longitude 102° 09'34,1"). *Bacillus* sp. strain JS4 bacterium was sourced from the sea water and registered on NCBI GenBank (Setiaji et al 2020). 12 liters of *Bacillus* sp. strain JS4 bacterium was cultured for 7 days, then extracted using ethyl acetate. The filtrate was then steamed using rotary evaporator at 40°C to a thick consistency. The extract was isolated using flash chromatography. The following sequence is how to get the compound: first extract ethyl acetate in a column using silica gel 60. Then it is eluted with mobile phase with polarization level of n-hexane: ethyl acetate (9:1 to 10:0), ethyl acetate: methanol (9:1 to 10:0) for 200 ml. The absorbance of the compound was measured with ultraviolet (UV) spectroscopy. The compound was dissolved in methanol (HPLC grade), then injected through the injection sample, the mobile phase (acetonitrile-MeOH) was to bring the sample to move through the chromatography column which was a stationary phase. FT-IR scpectroscopy was used to find the functional groups in the compound. 1 mg of the compound was crushed with KBr homogenously and the infrared absorbance was measured at wavelength of 4500-450 cm. 2 mg of pure compound was dissolved with $CDCL_3$ for the measurement of analytic NMR (Nuclear Magnetic Resonance) spectroscopy, then ^{1}H NMR at 500 MHz frequency and ^{13}C NMR at 125 MHz frequency.

Inhibitory activity of compound JS4-E-01. Compound JS4-E-01 was tried with *V. algynolyticus*, *A. hydrophila* and *P. aeruginosa* microbe microscopic organisms by agar dispersion strategy, paper plate with distance across of 6 mm was utilized (Feliatra et al 2018). The procedure is as per the following: 1 mL of inocullent microbe microorganisms (OD 600nm = 0.08-0.1) was moved to 15 mL fluid agar supplement media to be vaccinated at 50°C, then, at that point, homogenized and poured onto a Petri dish. After the media loaded up with refined microbe microscopic organisms cemented, oxytetracyclin antimicrobial paper circle was utilized at positive control. 30 µL of methanol was dropped onto a paper circle and was utilized as bad control. 1 mg/mL JS4-E-01 compound was broken down with methanol, then, at that point, 30 µL was dropped onto a paper circle, then it was incubated at 30°C for 24 hours. The restraint capacity of JS4-E-01 was estimated from the width of the straightforward zone conformed to the plate.

Statistical analysis. Information recorded was dissected spellbindingly by contrasting the size of the straightforward zone framed by the standards of the hindrance of the antibacterial compound.

Results and Discussion

Structure characterization. For the result of secondary metabolite of *Bacillus* sp. strain JS4 bacterium isolation, 3 mg of white crystal was collected and given the code JS4-E-01. The ultraviolet spectroscopy measurement on the JS4-E-01 compound shown maximum absorbance on wave 258 and 211 nm. The infrared spectrum on JS4-E-01 shown that there was absorbance ribbon on 3203 cm^{-1}, presence of the O-H functional group, 2896 cm^{-1}, presence of the C-H functional group, 1732 cm^{-1} presence of the C=O functional group, 1645 cm^{-1} presence of the C=C functional group, and 1236 cm^{-1} presence of the C-O functional group.

The characterization of JS4-E-01 using NMR (Nuclear Magnetic Resonance) spectroscopy with 500 MHz frequency for ^{1}H NMR and 125 MHz for ^{13}C NMR, using CD_3OD as a dissolvent. ^{1}H NMR spectrum showed that JS4-E-01 has 10 hydrogen atoms that is in chemical shift δ_H 7.39 (1H); 5.60 (1H); 3.37 (4H); 2.58 (4H). Spectrum of ^{13}C NMR showed that JS4-E-01 compound has 8 carbon atoms at the chemical shift δ_C 173.4; 167.3; 143.6; 101.7; 36.9; 31.3. At chemical shifts 7.39 and 5.60 shown double signal with one proton integration, the indication was the presence of protons from the double bond with J=7.6 Hz. This proton was bonded with a different C atom, which was at C_4 (164.3 ppm) and C_5 (143.6 ppm) (Table 1).

Table 1

Interpretation of H NMR and C NMR chemical shift of JS4-E-01 compound

No	*δ C (ppm)*	*δ H (ppm)*		
1	101.7	3.37 (t)	J = 6.8	(2H)
2	36.9	2.61 (t)	J = 6.9	(2H)
3	173.4	-		
4	164.3	7.39 (d)	J = 7.6	(1H)
5	143.6	5.60 (d)		(1H)
6	173.4	-		
7	31.3	2.58 (t)		(2H)
8	101.7	3.37 (t)		(2H)

According to the COSY spectrum it can be seen the correlation of H-1 (δ_H 3.37) with H-2 (δ_H 2.61); H-4 (δ_H 7.39) with H-5 (δ_H 5.60) and H-7 (δ_H 2.58) with H-8 (δ_H 3.37) (Figure 1).

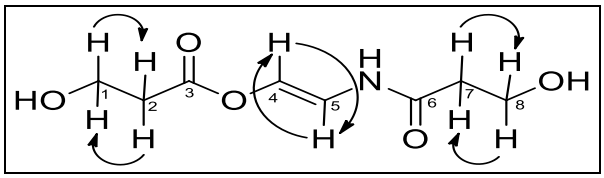

Figure 1. COSY correlation of JS4-E-01 compound.

Based on the infrared spectrum data analysis, NMR spectrum result, and COSY result, it can be identified that JS4-E-01 compound is N-[2-(3-hydroxypropanoyl)ethenyl]3-hydroxypropanamide (Figure 2).

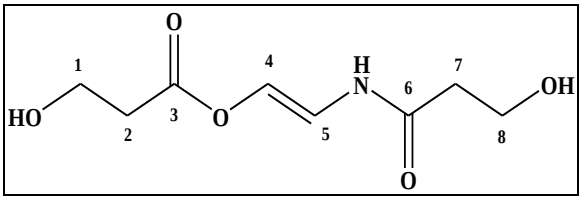

Figure 2. Structure of JS4-E-01 compound N-[2-(3-hydroxypropanoyl)ethenyl]3-hydroxypropanamide.

JS4-E-01 compound on pathogen bacteria. JS4-E-01 compound was a result of secondary metabolite isolation JS4 (*Bacillus* sp.) bacterium tested on pathogen bacteria which affects the fish ecosystem which are *V. alginolyticus*, *A. hydrophila* and *P. aeruginosa*. The inhibition ability of JS4-E-01 compound differed to each pathogen bacteria, it can be seen from the transparent zone around the paper disc that has been dropped with the compound. The transparent zone formed was caused by the activity of JS4-E-01 compound by pushing back the growth of the pathogen bacteria. The highest inhibition ability done by the JS4-E-01 was to *V. alginolyticus* for 13.3 mm (Table 2).

Table 2

Inhibition test of JS4-E-01 on pathogen bacteria

Compound	*Inhibition zone diameter (mm)*		
	A. hydrophila	*P. aeruginosa*	*V. alginolyticus*
JS4-E-01	10.5 ± 0.07	12.3 ± 0.35	13.3 ± 0.35

The better inhibition against the growth of pathogen bacteria was caused by the presence of functional groups O-H (hydroxyl), C=O (carbonyl), and C=C (double bond) that can inhibit the growth of microbes. Every compound was able to affect the antibacterial activity with the presence of activation and functional groups such as hydroxyl that caused the death to the bacteria. Fitriana and Prabawati (2018) stated that the hydroxyl functional group in the compound was able to create a toxic effect through organic component and nutritional transfer on the bacteria.

Voet and Voet (2010) stated that hydroxyl ion induction acted with cell membrane and component such as mitochondria that cause irreversible change inside the bacteria's structure. This caused the elimination of biological enzyme activity and disturbance in the cellular metabolism.

N-[2-(3-hydroxypropanoyl)ethenyl]3-hydroxypropanamide that was produced by heterotrophic bacterium *Bacillus* sp. strain JS4 was able to strongly inhibit the growth of pathogen bacteria. A number of research reported that *Bacillus* sp. bacteria were able to produce antimicrobial compounds. Xiu et al (2017) stated that *Bacillus* sp. bacteria were able to produce pumilacidin compounds. Pumilacidin has antibacterial activity toward gram negative pathogen bacteria.

Bacillus subtilis MTCC 10403 that was isolated from brown seaweed *Anthophycus longifolius* produced a compound that had potential as an antibacterial against *Vibrio parahaemolyticus*, *V. vulnificus,* and *Aeromonas hydrophila* pathogen bacteria (Chakraborty et al 2018). *Bacillus amyloliquefaciens* bacterium that was isolated from *Padina gymnospora* seaweed produced a compound that has antibacterial activity against *Aeromonas hydrophila*, *Vibrio harveyi*, *Vibrio vulnificus* and *Vibrio parahaemolyticus* bacteria (Chakraborty et al 2017). Zidour et al (2017), stated *Bacillus pumilus* bacterium produced amicoumacin compound which structurally has pharmacology characteristics such as antibacterial, anti-inflammatory, and antiulcer.

Conclusions. *Bacillus* sp. strain JS04 bacterium delivered N-[2-(3-hydroxypropanoyl)ethenyl]3-hydroxypropanamide compound. This compound had the option to repress the development of *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Vibrio alginolyticus*.

Acknowledgements. The creators are appreciative to the Laboratory of Biochemistry of the Faculty of Agriculture, Universitas Islam Riau, Pekanbaru, Indonesia and its associates for supporting this study.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Abdelhamed H., Ibrahim I., Baumgartner W., Lawrence M. L., Karsi A., 2017 Characterization of histopathological and ultrastructural changes in channel catfish experimentally infected with virulent *Aeromonas hydrophila*. Frontiers in Microbiology 8:1519.

Cai S., Cheng H., Pang H., Jian J., Wu Z., 2018 AcfA is an essential regulator for pathogenesis of fish pathogen *Vibrio alginolyticus*. Veterinary Microbiology 213:35-41.

Chaix G., Roger F., Berthe T., Lamy B., Bilak J. E., Lafite R., Leray F. J., Petit F., 2017 Distinct aeromonas populations in water column and associated with Copepods from estuarine environment (Seine, France). Frontiers in Microbiology 8:1259.

Chakraborty K., Thilakan B., Raola V. K., 2018 Previously undescribed antibacterial polyketides from heterotrophic *Bacillus amyloliquefaciens* associated with seaweed *Padina gymnospora*. Applied Biochemistry Biotechnology 184(2):716-732.

Chakraborty K., Thilakan B., Raola V. K., 2017 Antimicrobial polyketide furanoterpenoids from seaweed associated heterotrophic bacterium *Bacillus subtilis* MTCC 10403. Phytochemistry 142: 112-125.

Chen J., Sun R., Pan C., Sun Y., Mai B. X., Li Q. X., 2020 Antibiotics and Food Safety in Aquaculture. Journal of Agricultural and Food Chemistry 68(43):11908-11919.

Chen Y., Cai S., Jian J., 2019 Protection against *Vibrio alginolyticus* in pearl gentian grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatu*) immunized with an acfA-deletion live attenuated vaccine. Fish & Shellfish Immunology 86:875-881.

Feliatra, Muchlisin Z. A., Teruna H. Y., Utamy W. R., Nursyirwani, Dahliaty A., 2018 Potential of bacteriocins produced by probiotic bacteria isolated from tiger shrimp and prawns as antibacterial to *Vibrio*, *Pseudomonas* and *Aeromonas* species on fish. F1000 Research 7(415):1-18.

Fitriana, Prabawati S. Y., 2018 Synthesis of 3-acetyl-7-(hydroxy) Coumarin compounds and their activity against *Staphylococcus aureus* and *Shigella flexneri* bacteria. Indonesian Journal of Materials Chemistry 1(1):9-13.

Gellatly S. L., Hancock R. E. W., 2013 *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. Pathogens and Disease 67:159–173.

Gozari M., Alborz M., El-Seedi H. R., Jassbi A. R., 2021 Chemistry, biosynthesis and biological activity of terpenoids and meroterpenoids in bacteria and fungi isolated from different marine habitats. European Journal of Medicinal Chemistry 210:112957.

Hilliam Y., Kaye S., Winstanley C., 2020 *Pseudomonas aeruginosa* and microbial keratitis. Journal of Medical Microbiology 69:3–13.

Lee J., Zhang L., 2015 The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. Protein and cell 6(1):2641.

Liu W., Huang L., Su T., Qin Y., Zhao L., Yan Q., 2017 Contributions of the oligopeptide permeases in multistep of *Vibrio alginolyticus* pathogenesis. Microbiology Open 6(5):1-9.

Rameshkumar P., Nazar A. K. A., Pradeep M. A., Kalidas C., Jayakumar R., Tamilmani G., Sakthivel M., Samal A. K, Sirajudeen S., Venkatesan V., Nazeera B. M., 2017 Isolation and characterization of pathogenic *Vibrio alginolyticus* from sea cage cultured cobia (*Rachycentron canadum* Linnaeus 1766) in India. Letters in Applied Microbiology 65(5):423-430.

Rodriguez-Mozaz S., Vaz-Moreira I., Giustina S. V. D., Llorca M., Barcelo D., Schubert S., Berendonk T. U., Michael-Kordatou I., Fatta-Kassinos D., Martinez J. L., Elpers C., Henriques I., Jaeger T., Schwartz T., Paulshus E., O'Sullivan K., Pärnänen K. M.

M., Virta M., Do T. T., Walsh F., Manaia C. M., 2020 Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. Environment International 14:105733. https://doi.org/10.1016/j.envint.2020.105733.
Setiaji J., Feliatra F., Teruna H. Y., Lukistyowati I., 2020 Antibacterial activity in secondary metabolite extracts of heterotrophic bacteria against *Vibrio alginolyticus*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa*. F1000 Research 9:1491.
Souza C. F., Baldissera M. D., Descovi S. N., Zeppenfeld C. C., Camila M Verdi C. M., Santos R. C. V., da Silva A. S., Baldisserotto B., 2019 Grape pomace flour alleviates *Pseudomonas aeruginosa*-induced hepatic oxidative stress in grass carp by improving antioxidant defense. Microbial Pathogenesis 129:271-276.
Stratev D., Odeyemi O. A., 2016 Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review. Journal of Infection and Public Health 9(5):535-44.
Voet D., Voet J. G., 2010 Biochemistry. 4ed. New York: John Wiley & Sons, Inc. 1520 p.
Weiler A. J., Spitz O., Gudzuhn M., Schott-Verdugo S. N., Kamel M., Thiele B., Streit W. R., Kedrov A., Schmitt L., Gohlke H., Kovacic F., 2022 A phospholipase B from *Pseudomonas aeruginosa* with activity towards endogenous phospholipids affects biofilm assembly. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1867(4):159101.
Xiu P., Liu R., Zhang D., Sun C., 2017 Pumilacidin like lipopeptides derived from marine bacterium *Bacillus* sp. Strain 176 suppress the motility of *Vibrio alginolyticus*. Applied and Environmental Microbiology 83(12):1-14.
Zhang D., Moreira G. S., Shoemaker C., Newton J. C., Xu D. H., 2016 Detection and quantification of virulent *Aeromonas hydrophila* in channel catfish tissues following waterborne challenge. FEMS Microbiology Letter 363(9):1-5.
Zidour M., Chevalier M., Belguesmia Y., Cudennec B., Grard T., Drider D., Souissi S., Flahaut C., 2017 Isolation and characterization of bacteria colonizing *Acartia tonsa* copepod eggs and displaying antagonist effects against *Vibrio anguillarum, Vibrio alginolyticus* and other pathogenic strains. Frontiers in Microbiology 8(1919):1-13.

Received: 07 February 2022. Accepted: 23 May 2022. Published online: 19 February 2023.
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How to cite this article:
Setiaji J., Feliatra F., Teruna H. Y., Lukistyowati I., Heriyanto, 2023 Activity of an antibacterial compound produced by *Bacillus* sp. strain JS4 (MT102913.1). AACL Bioflux 16(1):591-596.