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Isolation and determination of *Vibrio* spp. pathogen from *Sciaenops ocellatus* suffering from hemorrhagic disease under cage culture in Vietnam

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KEYWORDS

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Cage culture

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Red drum

Sciaenops ocellatus

Vibrio

ABSTRACT

This study was carried out to isolate and determine the *Vibrio* spp. from the Red drum fish (*Sciaenops ocellatus*) suffering from the hemorrhagic disease in Vietnam. In this study, 18 strains of *Vibrio* bacteria were identified from 27 samples of Red drum fish. The isolated bacterial strains were identified with the *16S rRNA* sequencing method and checked for morphological, physiological, and biochemical characteristics by using the API 20E KIT. Results of the study revealed the presence of twelve strains of *V. alginolyticus*, three strains of *V. fluvialis*, and three strains of *V. orientalis*. All *Vibrio* strains have gene similarities with those on the Genbank ranging from 98.05 to 100%. The biochemical characteristics of these 18 isolates were similar and these are susceptible to tetracycline and doxycycline and entirely resistant to ampicillin, amoxicillin, and erythromycin.

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1 Introduction

Red drum (*S. ocellatus*) is a marine fish species with high economic value, commonly farmed in Texas and Florida, USA, to provide alternatives to wild-caught fish and stock enhancement efforts (FAO 2009). Red drums have been raising in Vietnam since 1999 and become the most popular species in fish culture due to high productivity (Ministry of Fisheries 2005). In Hue, it has been cultured since 2015 and is more favorable in cage culture in coastal and lagoon areas. Gibson et al. (2021) reported that various *Vibrio* spp. including *V. harveyi* and *V. carchariae* affected the fish cage culture and caused hemorrhagic diseases. Further, *V. vulnificus*, *V. brasiliensis*, *V. cholera*, and *V. parahaemolyticus* are also associated with the Red drums' hemorrhagic disease (Quang et al. 2020). Similarly, Deng et al. (2020) also reported that *Vibrio* is seriously associated with various infectious diseases in marine fish in South China, and these are mainly caused by *V. alginolyticus*, *V. azureus*, *V. fluvialis*, and *V. orientalis* species. In addition, *V. alginolyticus* and *V. parahaemolyticus* strains are also pathogens for the coastal aquaculture systems in Guangdong, China, and Taiwan (Xie et al. 2005). Jeong et al. (2020) indicated the current status and future directions of fish vaccines employing virus-like particles limited and must be integrated intervention to prevent bacterial multiplication. In another study, Cao et al. (2017) also stated that the development of VaBGs (*V. alginolyticus* BGs) like vaccine causes a stronger humoral and cellular immune response and protects mice and fish from *V. alginolyticus*, and found that VaBGs is superior to the conventional FKC vaccine (Formalin-killed whole-cell vaccine). Even *V. alginolyticus*, *S. iniae*, and *Photobacterium damselae* have also been identified as the causative agent of hemorrhagic disease, ulcers, fin erosion, and blindness in Cobia (*Rachycentron canadum*) (Trung and Dung 2018). The aim of this study was to isolate and identify *Vibrio* spp. from the Red drum (*S. ocellatus*) fishes suffering from hemorrhagic disease under cage farming in Thua Thien Hue, Vietnam, and to provide basic data on biochemical characteristics and antibiotic resistance in *Vibrio* bacteria.

2 Material and Methods

2.1 Sampling and preparation

Thirty-five specimens of Red drums with hemorrhage signs were collected from the four culture cage sites in Hai Duong commune, Huong Tra, Thua Thien Hue, Vietnam. These samples were collected between January to March 2020. The geographic coordinates of sampling sites are: (1) 107°36'39.3984" East and 16°34'0.558012" North; (2) 107°36'51,89544" East and 16°33'57.93696" North; (3) 107°37'3.20988" East and 16°33'58.190796" North; and (4) 107°37'15.12192" East and 16°33'59.01696" North (Figure 1). After collection, the live fish were transported by styrofoam boxes (with manual aerator, temperature from 15 to 18°C) to the laboratory for bacterial isolation and analysis of genes and others.

2.2 Physical and chemical characteristics of water

The pH (HANNA HI98107 pH handheld pH meter, Romania), temperature (mercury thermometer), salinity (refractometer), and dissolved oxygen (sera test KIT, Virtue) of the sampling sites water samples were also recorded.

2.3 Bacteria isolation and identification

The fish were washed thoroughly under tap water and dried. The outer surface of the fish was disinfected with 70% ethanol, and the fish was opened with a sterilized scalpel. The internal pathological signs were recorded and the damaged tissues from the brain, liver, kidney, and spleen were separated with sterilized culture rods. Extract sticks on the culture rod were inoculated on the thiosulfate citrate bile salts sucrose medium (TCBS, Himedia, India) and cultured at 28°C for 24 hours. The prevalent and loose colonies were further cultured in the TCBS medium under the same conditions for total DNA extraction.

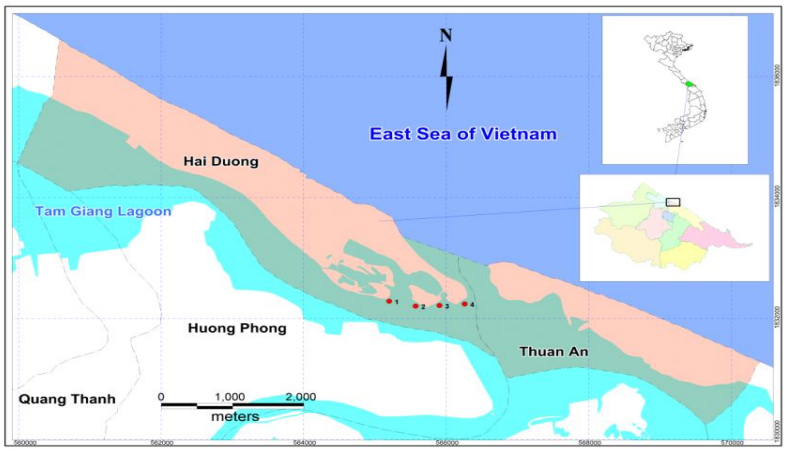

Figure 1 Geographical distribution of the four Sampling sites

2.4 Molecular identification of *Vibrio*

2.4.1 Total DNA extraction

The *Vibrio* cell lines isolated from Red drum fish with hemorrhagic signs were grown proliferatively on the trypto-casein soy broth (TSB) supplemented with 2% NaCl and shaken at 180 rpm at 30°C for 24 hours. Cell biomass was obtained by centrifugation at 8,000 rpm/min for 2 min at 4 °C. The total DNA of *Vibrio* cell lines was extracted by the modified phenol/chloroform method with some modification and the bacterial cells were directly extracted with phenol SDS/lysozyme or proteinase K (Neumann et al. 1992).

2.4.2 *16S rRNA* gene amplification with PCR and nucleotide sequence analysis

The *16S rRNA* gene regions of bacterial strains were amplified by using PCR with specific primer pairs of 27F "AGAGTTTGATCMTGGCTCAG" and 1492R "TACGGYTACCTTGTTACGACTT" (Gibson et al. 2021). The PCR was performed on the Applied Biosystems Life Technologies Thermo Fisher Scientific USA systems; the reaction components and thermal cycle used in this study are presented in Table 1.

The PCR product was subject to electrophoresis on 1% agarose gels stained with ethidium bromide, and the electrophoretic images were analyzed with a DyNA Light, Dual-Intensity UV Transilluminator system. This was followed by the purification and sequencing of *16S rRNA* gene region by Sanger method on an ABI PRISM® 3100 Avant Genetic Analyzer (Applied Biosystems) system by Maccrogen Company, Korea. The nucleotide sequence of the genomic region was determined and aligned by using the program Clustal-X (Thompson et al. 1997) and edited by using the BioEdit 7.0.5 software (Hall 1999). The nucleotide sequences of the genomic region were compared with the *16S rRNA* sequences of the microorganisms published on the World GenBank (GenBank) with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi).

2.5 Physiological and Biochemical characterization

The physiological and biochemical characteristics including Gram staining, oxidase, catalase, oxidase fermentation, bacterial motility was also estimated in the current study. The bacterial motility was determined with the hanging drop method and hemolysis by culturing on agar supplemented with 5% horse blood by Buller's method (Buller, 2004). The bacterial strains were tested by using the API 20E KIT (Bio-Mérieux, France) following the manufacturer's instructions. The reactions were carried out at 28°C, and the results were recorded after 24 hours.

In addition, several biochemical reactions were also carried out to fully understand the characteristics of the isolated *Vibrio* strains. The Voges-Prosakauer test and the fermentation of carbohydrates with the purple Broth Base (Difco, UK) supplemented with 5% glucose, fructose, galactose, glucose, glycerol, maltose, mannose was carried out (Phuoc 2014). The viability of *Vibrio* strains at different salt concentrations was conducted on the TSB medium supplemented with 0, 1, 6, 8, and 10% NaCl. The *Vibrio* strains isolated in this study were compared with those isolated by Buller (2004).

2.6 Antibiotic susceptibility test

The antibiotic susceptibility of *Vibrio* spp. was tested with the agar plate diffusion method. The *Vibrio* spp. strains were grown proliferatively on the TSB medium supplemented with 2% NaCl and inoculated at 28°C for 24 hrs in a culture cabinet with a shaking speed of 150 rpm. Then, the bacterial cell density was determined by determining the optical density (OD) at 600 nm on a UV-VIS spectrophotometer (U2900, Hitachi, Japan). The preparation was diluted to a concentration corresponding to OD 1 and further reach to 10^6 CFU/mL for the following experiments (Adnan et al. 2013).

One hundred micro-Littre of the 10^6 CFU/mL bacterial solution was evenly spread on the Mueller Hinton Agar medium (MHA, Himedia, India), supplemented with 2% NaCl, and dried at

Table 1 Specific PCR components and thermal cycles for *16S rRNA* gene region amplification

PCR components		Thermal cycle of PCR for *16S rRNA* gene amplification		
Element	Volume (µL)	Temperature (°C)	Time	Cycle
2× PCR master mix (2.4 mM dNTP each, 0.3 units Taq DNA polymerase)	25	95	5 min	1
10 pmol of 27F primer	1	95	1 min	30
10 pmol/µL of 1492 primer	1	57	50 s	
DNA (50 ng/µL)	1	72	1 min	
Sterile distilled water	22	72	10 min	Lengthen
Total	50	4	Storage temperatures	

ambient temperature for 30 minutes. This was followed by the inoculation of antibiotics discs on the media with the help of sterilized forceps. The antibiotics used in the study are tetracycline 30 ug (Te), amoxicillin 10 ug (Ax), neomycin 30 ug (Ne), ampicillin 10 ug (Am), kanamycin 30 ug (Kn), doxycycline 30 ug (Dx), enrofloxacin 5 ug (Ef), erythromycin 15 ug (Er), and cefotaxime 30 ug (Ct). The plates were incubated in an incubator at 28°C. The diameter of the sterile ring (inhibition zone) was measured after 24 hours.

The bacteria's antibiotic susceptibility or resistance to antibiotics was evaluated from the diameter of the sterile ring followed by the standards of the Clinical and Laboratory Standards Institute, 2018 (Sahu et al. 2018).

2.7 Statistical analysis

Database of water environment, infection rate, and physical characteristics were processed by Microsoft Excel version 2016. Further, *16S rRNA* gene region analysis was carried out with the help of MEGA X software.

3 Results and Discussion

3.1 Physical, chemical water parameters and sample characteristics

Physical and chemical characteristics of water such as pH, temperature, salinity, and dissolved oxygen have been represented in table 2 and the values of the tested parameters are within the acceptable range for nurturing Red drums. Out of 35 collected samples, 27 (77.14%) are showing various infection or disease symptoms. The diseased fish shows various symptoms such as tail amputation, hemorrhaging in gills, body, and skin, and fluid accumulation in the abdominal cavity. In general, environmental factors of the four sampling sites showed small changes and were suitable for Red drum culture, compared with marine water, the water body in the lagoon system a less than salinity.

The collected fish samples have weight ranges from 24.31 to 24.99 g with a length between 11.65 and 15.21 cm (Table 3). The fish body was opened and the internal organs were taken out for testing and checked disease symptoms.

Table 2 Physical and chemical characteristics of the examined sampling sites

Parameter	1		2		3		4	
	Mean ± SD	Min-Max	Mean ± SD	Min–Max	Mean ± SD	Min-Max	Mean ± SD	Min–Max
pH	7.95 ± 0.19	7.8–8.2	7.75 ± 0.31	7.5–8.2	7.83 ± 0.21	7.6–8.1	7.75 ± 0.24	7.5–8.0
T (° C)	26.40 ± 0.64	25.5–27.0	26.85 ± 1.01	26.0–28.0	26.23 ± 1.32	25.0–27.0	26.40 ± 1.14	25–27.6
Salinity (‰)	26.75 ± 4.27	21.0–30.0	25.00 ± 2.16	23.0–28.0	28.00 ± 1.41	27.0–30.0	28.75 ± 0.96	28–30
DO (mg/L)	6.00 ± 0.41	5.5–6.5	6.13 ± 0.25	6.0–6.5	6.38±0.48	6.0–7.0	6.63 ± 0.85	5.5–7.5

Table 3 Fish infection rate and physical characteristics

Point	Samples	Infection rate (%)	Length (cm)	Weight (g)
1	10	80.00	11.65 ± 0.84	24.77 ± 1.13
2	7	71.00	15.21 ± 1.65	26.14 ± 1.57
3	6	66.67	12.13 ± 0.55	24.31 ± 1.16
4	12	83.33	13.89 ± 1.64	24.75 ± 1.43
Total	35	77.14	13.22 ± 1.32	24.99 ± 0.56

Table 4 Ratio of bacterial strains isolated from various organs of hemorrhaged Red drums fish cultured in Thua Thien Hue province

Organ of isolation	Number of colonies	Ratio (%)
Brain	2	11.11
Liver	7	38.89
Kidney	5	27.78
Spleen	4	22.22
Total	18	100

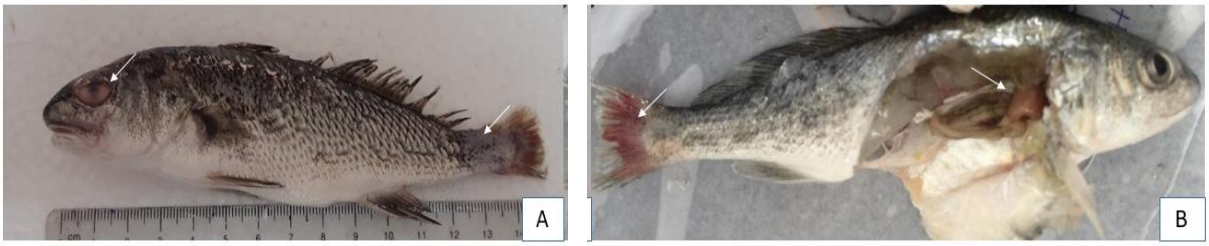

Figure 2 Characteristic signs of hemorrhagic disease are red spots on fins, torn tail, and hemorrhage (arrows, Figures A & B); mucus accumulation in the abdominal cavity (arrow, Figure B)

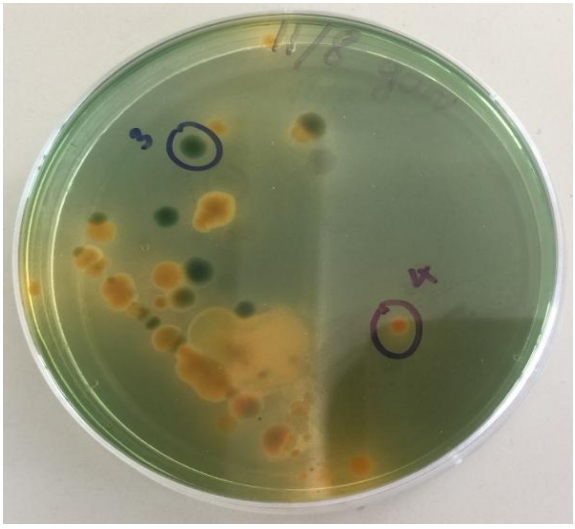

Figure 3 Bacterial colonies isolated on TCBS medium

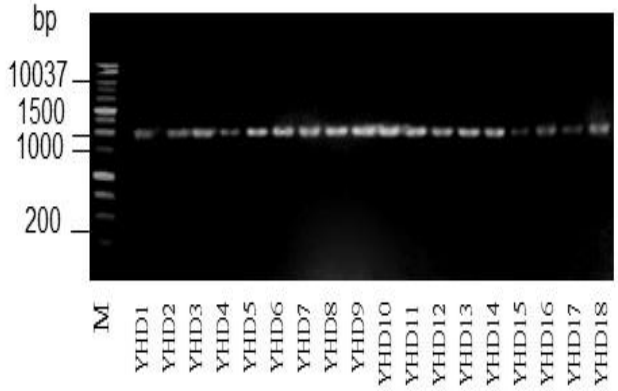

Figure 4 Electrophoresis of PCR product *16S rRNA*
M: DNA mass scale (Hyper Ladder™ 1 kb (200 bp to 10,037 bp), Bioline, Meridian Bioscience

3.2 Isolation and identification of the bacteria

In hemorrhaged Red drums, blue and yellow colonies of *Vibrio* spp. appeared on the TCBS medium after 24 h of culture (Figure 2 & 3). Different parts of the fish body have different levels of infection, and it was reported from 11.11% in the brain to 38.89% in the liver (Table 4), these results suggested higher *Vibrio* infection into the liver as compared to the other organs. However, each organ has a different resistance and needs to be tested separately with nucleotide sequence analysis of the *16S rRNA* gene region (table 4).

3.3 Molecular identification and phylogenetic tree generation

3.3.1 PCR product electrophoresis

PCR amplification of *16S rRNA* regions of all 18 bacterial strains isolated from hemorrhaged Red drums collected from all study sites gives a single band with an amplification rate of 100%. All PCR products of bacterial strains are highly concentrated and sharp. The size of the PCR product is approximately 1,500 bp, consistent with the original expected size (Figure 4). Electrophoresis of *16S rRNA* PCR product and M for DNA mass scale gave 200 bp to 10,037 bp yield (Hyper Ladder™ 1 kb, Bioline, Meridian Bioscience).

3.3.2 Nucleotide sequence of the *16S rRNA* gene region

The nucleotide sequence analysis of the *16S rRNA* gene region of the bacterial strains shows that the PCR amplification rate and the successful nucleotide sequence analysis rate are 100% equal. The sequence edited with the Bioedit software showed the gene regions sized from 1381 to 1448 bp with a mean at 1441 bp. The occurrence rate of each type of nucleotide in the region revealed that the guanine (G) occupies the first position with 31.86% percentages, this was followed by the adenine (25.19%), cytosine (22.31), uracine (20.64), and prevalence (G + C) 54.17% (Table 5).

The *16S rRNA* gene region analysis with the MEGA X software showed that the conserved area for bacterial populations isolated from Red drums has 83/1448 modified nucleotide positions, accounting for 5.73% of the total length of the gene (Table 6).

Table 5 Nucleotide sequence analysis of each *16S rRNA* gene region (%)

No.	Sample code	(U) (%)	C(%)	A(%)	G(%)	G + C(%)	Gene length (bp)
1	YHD1	20.64	22.59	25.34	31.43	54.02	1381
2	YHD2	20.57	22.29	25.28	31.86	54.16	1444
3	YHD3	20.51	22.31	25.35	31.84	54.14	1448
4	YHD4	20.86	22.24	24.93	31.98	54.21	1448
5	YHD5	20.53	22.33	25.10	32.04	54.37	1442
6	YHD6	20.86	22.24	24.93	31.98	54.21	1448
7	YHD7	20.72	22.25	25.16	31.88	54.12	1443
8	YHD8	20.51	22.31	25.35	31.84	54.14	1448
9	YHD9	20.86	22.24	24.93	31.98	54.21	1448
10	YHD10	20.72	22.25	25.16	31.88	54.12	1443
11	YHD11	20.57	22.29	25.28	31.86	54.16	1444
12	YHD12	20.76	22.43	25.35	31.46	53.89	1440
13	YHD13	20.55	22.35	25.19	31.90	54.26	1445
14	YHD14	20.55	22.35	25.19	31.90	54.26	1445
15	YHD15	20.57	22.29	25.28	31.86	54.16	1444
16	YHD16	20.53	22.33	25.10	32.04	54.37	1442
17	YHD17	2051	22.31	25.35	31.84	54.14	1448
18	YHD18	20.72	22.25	25.16	31,88	54.12	1443
	Mean	20.64	22.31	25.19	31.86	54.17	1441

Table 6 Some characteristics based on *16S rRNA* gene regions of bacterial strains in PCR populations

PCR success rate (%)	Sequencing success rate (%)	Total genomic length (bp)	Percentage of polymorphic nucleotide sites (%)
100	100	1381–1448	5.73

3.3.3 Molecular identification

The BLAST results were compared with the world Genbank and reported that 18 bacterial strains belong to the genus *Vibrio*, and among the various *Vibrio* species, *V. alginolyticus* showed the most significant occurrence (67%), this was followed by *V. fluvialis* and *V. orientalis* with the same occurrence rate (17%). The similarities range was reported between 98.05 to 100%. All nucleotide sequences of the bacterial strains are registered on the NCBI Genbank (Genbank Database) with corresponding reference codes (Table 7).

Vibrio has been identified as a common pathogen in marine fish species and causing great damage to many marine fish species of economic value (Deng et al. 2020).

This study reported 12 *V. alginolyticus* strains, 3 *V. Fluvialis* strains, and 3 *V. orientalis* strains (Table 7). Among the reported *Vibrio* spp., *V. alginolyticus* is considered one of the most harmful species for aquatic animals (Deng et al. 2020). Results of the current study are in agreement with the findings of Rameshkumar et al. (2017) those who reported *V. alginolyticus* as the most destructive species for Cobia (*Rachycentron canadum*) under Indian cage cultured aquaculture. Similarly, Dahanayake et al. (2018) isolated a total of 41 *Vibrio* spp., including of 23 *V. alginolyticus*, 11 of *V. fluvialis*, and 7 of *V. antiquarius* from Oyster (*Crassostrea giga*).

3.4 Biochemical Characteristics of isolated bacterial strains

The 18 bacterial strains identified in Table 7 were subjected to their morphological, physiological, and biochemical characterization (Figure 5, Table 8).

The physiological and biochemical test with the API 20E KIT (Bio Mérieux, France) indicates that among the 18 strains, 12 strains

Table 7 Percentage similarities of isolates based on *16S rRNA* gene sequencing published in GenBank

No	Isolated	Genbank code	GenBank reference	Similarity (%)
1	*V. alginolyticus* strain, YHD1	MZ753696	MH298564.1	98.05
2	*V. alginolyticus* strain, YHD3	MZ753698	CP051109.1	99.59
3	*V. alginolyticus* strain, YHD5	MZ753700	MN843961.1	99.72
4	*V. alginolyticus* strain, YHD7	MZ753702	MN938185.1	99.86
5	*V. alginolyticus* strain, YHD8	MZ753703	CP051109.1	99.59
6	*V. alginolyticus* strain, YHD10	MZ753705	MN938185.1	99.86
7	*V. alginolyticus* strain, YHD12	MZ753707	MH298564.1	98.05
8	*V. alginolyticus* strain, YHD13	MZ753708	MN938360.1	99.65
9	*V. alginolyticus* strain, YHD14	MZ753709	MN938360.1	99.65
10	*V. alginolyticus* strain, YHD16	MZ753711	MN843961.1	99.72
11	*V. alginolyticus* strain, YHD17	MZ753712	CP051109.1	99.59
12	*V. alginolyticus* strain, YHD18	MZ753713	MN938185.1	99.86
13	*V. fluvialis* strain,YHD4	MZ753699	CP051126.1	100
14	*V. fluvialis* strain, YHD6	MZ753701	CP051126.1	100
15	*V. fluvialis* strain, YHD9	MZ753704	CP051126.1	100
16	*V. orientalis* strain,YHD11	MZ753706	MN945276.1	100
17	*V. orientalis* strain,YHD2	MZ753697	MN945276.1	100
18	*V. orientalis* strain,YHD15	MZ753710	MN945276.1	100

Genbank registration Code No: https://submit.ncbi.nlm.nih.gov/subs/?search=SUB10184933

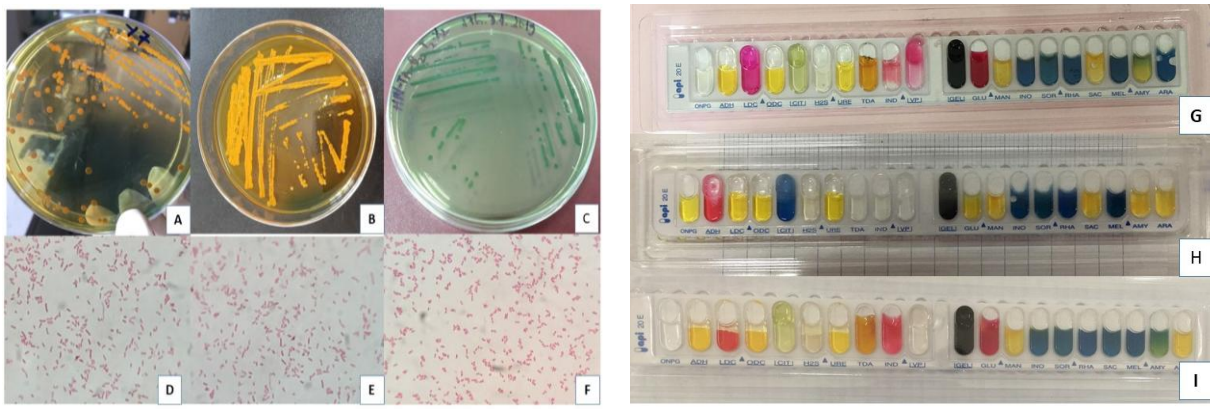

Figure 5 Colony appearance on TCBS: (A) *V. alginolyticus*; (B) *V. fluvialis*; (C) *V. orientalis*; Gram staining (D) *V. alginolyticus*; (E) *V. fluvialis*; (F) *V. orientalis*; identification of bacteria isolated from Red drums with API 20E KIT: (G) *V. alginolyticus*; (H) *V. fluvialis*; (I) *V. orientalis*

showed 414725, 3 strains showed 3246126, and 3 strains showed 4066106 responding to *V. alginolyticus*, *V. fluvialis***,** and *V. orientalis* respectively (Buller 2004). Among the reported strains, *V. alginolyticus* strain shows comma-shaped yellow colonies on the TCBS medium and belonging to the Gram-negative bacteria group (Figure 5 A & D). Further, this strain is indole unproductive, capable of degrading nitrate, producing catalase, oxidase, and positive Voges–Proskauer reaction. *V. alginolyticus* strain can also ferment glucose, sucrose, mannitol, sorbitol, and arabinose. One of these strains is capable of producing H_2S (11/12). The physiological and biochemical parameters results of *V. alginolyticus* isolated from diseased Red drums are homogenous with *V. alginolyticus* ATCC33787 strains isolated from diseased Oysters (Escalona et al. 2006).

Table 8 Physiological and biochemical parameters of *Vibrio* spp. isolated from diseased Red drums

No.	Indicator		*V. alginolyticus* (Buller, 2004)	*V.alginolyticus* *n* = 12	*V. fluvialis* (Buller, 2004)	*V. fluvialisn* = 3	*V. orientalisn* = 3 (Buller, 2004)	*V. orientalisn* = 3
1	Gram staining		–	–	–	–	–	–
2	Form		Comma	Comma	Curved rod	Curved rod	Curved rod	Curved rod
3	Colonies on TCBS		Yellow	Yellow	Yellow	Yellow	Green	Green
4	Colonies on TSA		ND	Milky white	Milky white	Milky white	ND	Milky white
5	Growth in NaCl solution (%)	0	–	–	–	–	–	–
		1	ND	+	+	+	+	+
		6	+	+	+	+	+	+
		8	+	+ (10/12)	–	–	+	+
		10	–/+	–/+	–	–	–	–
6	API 20E		414725	414725	3246126	3246126	4066106	4066106
7	Oxidase		+	+	+	+	+	+
8	Catalase		+	+	+	+	+	+
9	H_2S synthesis		–	– (11/12)	–	–	–	–
10	NO_3 decomposition		+	+	+	+	+	+
11	Indol		–	–	+	+	+	+
12	Voges–Proskauer		+	+	–	–	–	–
13	Citrate use		+	+	+	+	–	–
14	Glucose		+	+	+	+	–	–
15	Mannitol		+	+	+	+	+	+
16	Sorbitol		+	+	–	–	–	–
17	Sucrose		+	+	+	+	–	–
18	Arabinose		–	–	+	+	+	+

Among the eighteen bacterial strains three (16.67%) belonging to the Gram-negative *V. fluvialis*, and have curved rod-shaped morphology and yellow colonies on the TCBS medium (Figure 5 B & E). *V. fluvialis* has oxidase-positive nature, capable of producing catalase and indole, fermenting glucose, mannitol, sucrose, and arabinose but not sorbitol. Further, rest three (16.67%) bacterial strains were identified as *V. orientalis* strains and these have blue colonies on the TCBS medium. *V. orientalis* appeared as short curved rods and belong to the group of Gram-negative bacteria (Figure 5 C & F). *V. orientalis* can grow at different NaCl concentrations (1, 6, and 8%) and showed oxidase, catalase, mannitol, and arabinose positive and glucose, sorbitol, sucrose, and H_2S-negative reaction. *V. fluvialis* and *V. alginolyticus* are well known for carrying out pathogenic factors for aquatic animals (Ramamurthy et al. 2014). Meanwhile, *V. orientalis* belongs to the Orientalis clade in *Vibrio* genus in which *V. tubiashii* and *V. sinaloensis* have been reported causing diseases for bivalves and fishes (Hada et al. 1984; Gomes et al. 2018). Moreover, Restrepo et al. (2018) reported a *V. punensis* which is closely related to *V. orientalis*, carrying the toxic genes which cause disease in shrimp. Due to limited information regarding *V. orientalis* strain, this strain is not yet recognized as pathogenic *Vibrio* species for aquatic animals. Thus, further investigations are required to establish the pathogenic characteristics of *V. orientalis*.

3.5 Antibiotic susceptibility of *Vibrio* strains

All 18 strains of *Vibrio* are entirely resistant to ampicillin, amoxicillin, and erythromycin (Table 9). Similarly, the Am resistance in *Vibrio* strains isolated from the marine environment was reported by Stabili et al. (2010). Trung and Dung (2018) also reported antibiotic resistance against ampicillin, streptomycin, and erythromycin in *V. alginolyticus* causing disease in the caged Cobia (*R.canadum*) cultured in Kien Giang, Vietnam.

Table 9 Antibiotic susceptibility rate of *Vibrio* strains isolated from diseased Red drums

Antibiotics	Rate (%)								
	V. alginolyticus			*V. fluvialis*			*V. orientalis*		
	S	I	R	S	I	R	S	I	R
β-lactams									
Cefotaxime (Ct, 30 ug)	75	25	0	66.7	33.3	0	66.7	33.3	0
Ampicillin (Am, 10 ug)	0	0	100	0	0	100	0	0	100
Amoxicillin (Ax, 10 ug)	0	0	100	0	0	100	0	0	100
Quinolones									
Enrofloxacin (Ef, 5 ug)	66.7	33.3	0	66.7	33.3	0	66.7	33.3	0
Aminoglycosides									
Kanamycin (Kn, 30 ug)	50	33.3	16.7	66.7	33.3	0	66.7	33.3	0
Neomycin (Ne, 30 ug)	41.7	33.3	25	33.3	33.3	33.3	33.3	33.3	33.3
Macroid									
Erythromycin (Er, 15ug)	0	0	100	0	0	100	0	0	100
Tetracyclines									
Tetracycline (Te, 30 ug)	100	0	0	100	0	0	100	0	0
Doxycycline (Dx, 30 ug)	100	0	0	100	0	0	100	0	0

S: Sensitive; I: Intermediate; R: Resistant

All isolates are found sensitive to tetracycline and doxycycline. Tetracycline is an antibiotic allowed in aquaculture, so farmers often use it to prevent and treat the diseases caused by bacteria in fish and shrimp farms. These findings are in agreement with the findings of Trung and Dung (2018). Tetracycline has long been the most commonly used antibiotic in the Korean aquaculture system, especially for the species severely infected by *Vibrio*. According to Hoa et al. (2019), *V. alginolyticus* strains cause disease in sea bass (*Lates calcarifer*) in Vietnam, and these strains are sensitive to doxycycline, erythromycin, nalidixic acid, oxolinic acid, oxytetracycline, streptomycin, sulphonamide, and tetracycline but resistant to ampicillin and neomycine. Table 9 indicated that all 12 strains of *V. alginolyticus* have sensitivity to cefotaxime (75%), enrofloxacin (66.7%), kanamycin (50%), and neomycin (41.7%); but there were 3 strains of *V. fluvialis* and *V. orientalis* are sensitive to cefotaxime (66.7%), enrofloxacin (66.7%), kanamycin (66.7%), and neomycin (33.3%). Kang et al. (2016) reported that 15 strains of *V. alginolyticus* isolated from oysters are completely resistant to ampicillin, highly sensitive to tetracycline (100%); cefotaxime (86.7%), and kanamycin (73.3%), and moderately resistant to erythromycin.

Conclusions

Results of the current study reported the presence of a total of 18 *Vibrio* strains from the hemorrhaged Red drums cultured in cages. Among the reported 18 strains, 12 strains were of *V. alginolyticus*, 3 of *V. fluvialis*, and 3 of *V. orientalis*. The results from this study also confirmed that the *Vibrio* spp. are generally homogeneous in biochemical characteristics. Further, isolated *Vibrio* strains have *16S rRNA* gene homology ranging from 98.05 to 100% and correspond to codes 4147125, 3246126, and 4066106 on the GenBank (https://csdlkhoahoc.hueuni.edu.vn/index.php/certificate/edit/id/16). These *Vibrio* strains are susceptible to tetracycline and doxycycline and resistant to ampicillin, amoxicillin, and erythromycin.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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