Current Microbiology (2022) 79:181
https://doi.org/10.1007/s00284-022-02879-5

Accurate Identification of Diverse N-acyl Homoserine Lactones in Marine *Vibrio fluvialis* by UHPLC-MS/MS

Jingjiao Bao[1,3] · Dengkang Guo[1,4] · Lei Jin[1,2,3] · Tiejun Li[1,3] · Hui Shi[1]

Received: 26 January 2022 / Accepted: 12 April 2022 / Published online: 5 May 2022

Abstract

Vibrio fluvialis is a marine opportunistic pathogen that frequently causes diseases in aquatic animals and humans. *V. fluvialis* can produce quorum sensing signaling molecules to coordinate cell density-dependent behavioral changes, including N-acyl homoserine lactone (AHL), which acts as a vital mediator of virulence-associated gene expression. Currently, several AHL molecules in *V. fluvialis* have been detected via biological and physicochemical methods, although different detection approaches have generated diverse AHL profiles. Here, we describe the AHL-producing bacterium, *V. fluvialis* BJ-1, which was isolated from marine sediments from the East China Sea. *V. fluvialis* BJ-1 could stimulate AHL-mediated β-galactosidase synthesis of the biosensor *Agrobacterium tumefaciens* NTL4 (pZLR4) but could not induce violacein production in the AHL reporter strain, *Chromobacterium violaceum* CV026. This bacterial isolate exhibited strong AHL-producing activity at low cell density; however, the AHL activity declined when population density remained at high levels. Analysis of the AHLs by Ultra-High-Performance Liquid Chromatography tandem Mass Spectrometry demonstrated that *V. fluvialis* BJ-1 produced five different AHL signaling molecules, including two linear chain AHL products (C_8- and C_{10}-HSL), and three β-carbon-oxidative AHL products (3-O-C_8-, 3-O-C_{10}- and 3-O-C_{12}-HSL). Significantly, the present study is the first to accurately define the AHL profile of marine *V. fluvialis*. In future, the coupling of UHPLC to ESI–MS/MS is expected to be utilized for the accurate determination of AHL profiles in marine *Vibrio*.

Introduction

Quorum sensing (QS) is an inter-bacterial communication process that enables bacteria to monitor and respond to changes in cell population density by regulating gene expression and the subsequent physiological responses [1, 2]. This cell-to-cell communication is mediated by small signal molecules called autoinducers (AIs), which are first synthesized by signal synthases in the cytoplasm and then directly penetrate the cell membrane or are transported via active efflux pathways into the surrounding environment [3]. When the accumulation of signal molecules in the environment increases to a sensing threshold level, the specific AIs signal binds to the receptor proteins that in turn regulate the expression of functional genes [4, 5]. In Gram-negative bacteria, the best characterized QS signal molecule is N-acyl homoserine lactone (AHL). AHL has been shown to be involved in the regulation of various phenotypes, such as motility, biofilm formation, bioluminescence, exoenzyme secretion, and virulence [6–8].

The amphipathic AHL molecule structure contains a hydrophilic homoserine lactone ring (HSL) and a hydrophobic acyl side chain. AHL diversity depends on the number of carbon atoms (4–18) on the acyl side chain, the substituent group (-H, -OH or -oxo) at the β-position, the methyl branch, and the level of saturation [9, 10]. The detection of AHL molecules is commonly achieved by biological and physicochemical methods. Microbiosensor-based methods are popular biological techniques for AHL screening owing to its low cost, high sensitivity, and an ability to detect a wide range of AHLs [11]. However, biosensor strains cannot accurately

✉ Lei Jin
jinlei2388@126.com

1 Zhejiang Marine Fisheries Research Institute, Zhoushan 316021, Zhejiang Province, China

2 Key Laboratory of Sustainable Utilization of Technology Research for Fishery Resource of Zhejiang Province, Zhoushan 316021, Zhejiang Province, China

3 Zhoushan Fishery Environments and Aquatic Products Quality Monitoring Center of Ministry of Agriculture China, Zhoushan 316021, Zhejiang Province, China

4 School of Life Science, Jiangsu Normal University, Xuzhou 221116, Jiangsu Province, China

classify the AHL molecules but instead exhibit a specific AHL production phenotype, making them more suitable for preliminary AHL screening. Various physicochemical detection methods are broadly applied to AHL identification and characterization, including thin-layer chromatography (TLC) [12], high-/ultra-high-performance liquid chromatography tandem mass spectrometry (HPLC/UHPLC-MS) [13–15], UHPLC-diode array detector-quadrupole time-of-flight mass spectrometer (DAD-QTOFMS) [16], gas chromatography tandem MS (GC–MS) [17], and nuclear magnetic resonance (NMR) [18]. Among them, UHPLC-MS or UHPLC-DAD-QTOFMS is increasingly used as a highly efficient physicochemical approach to the detection of AHLs and provides accurate determination of AHL types and concentrations, even available for the characterization of uncommon AHL molecules with ultra-long side chains or covalent double bonds [19, 20].

The genus *Vibrio* has a wide distribution in the marine environment and estuarine ecosystems worldwide [21], and some species are pathogenic to marine fauna and humans [22]. Many *Vibrio* species are reported to use QS to regulate various physiological functions critical to bacterial survival and pathogenesis [23]. For instance, the LuxI/LuxR system in *Vibrio fischeri* induces bioluminescence and has been used as the AHL-based QS model in Gram-negative bacteria [24]. One pathogenic *Vibrio* spp., the halophilic Gram-negative *Vibrio fluvialis*, can be found widely in marine environments and infects many aquatic animals and is thus responsible for huge economic losses in the aquaculture industry [25]. *V. fluvialis* is also an emerging foodborne pathogen and has been implicated in numerous human diseases, such as gastroenteritis, bacteremia, acute otitis, peritonitis, and hemorrhagic cellulitis with cerebritis and endophthalmitis [26, 27]. Two QS systems, based on AHL and CAI-1/AI-2 signal molecules, have been identified in *V. fluvialis* [28]. Several previous studies have shown that *V. fluvialis* strains are able to produce multiple AHL molecules [28–30], and that the AHL profiles identified using different detection approaches were diverse [23]. Nonetheless, the evidence for a diverse panel of AHL compounds produced by the pathogen *V. fluvialis* remains insufficient. In this study, the AHL signal molecules in *V. fluvialis* BJ-1 were accurately detected using the UHPLC-MS/MS method. Moreover, our present data provide an accurate identification and structure determination method for AHLs in marine *V. fluvialis*.

Materials and Methods

Chemicals, Medium, and Strains

Nine AHL standards (C_4-HSL, C_6-HSL, C_8-HSL, C_{10}-HSL, C_{12}-HSL, C_{14}-HSL, 3-oxo-C_8-HSL, 3-oxo-C_{10}-HSL, and 3-oxo-C_{12}-HSL) were purchased from Sigma-Aldrich Chemical Co. (Shanghai, China), dissolved in methanol and prepared to different concentrations before being stored at − 20 °C. All antibiotics (kanamycin, gentamicin, and ampicillin) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Luria–Bertani (LB) medium (yeast extract 5.0 g/L, NaCl 10.0 g/L, tryptone 10.0 g/L) and thiosulfate citrate bile salts sucrose agar (TCBS) were obtained from Qingdao Haibo Biotechnology Co., Ltd. CHROMagar *Vibrio* colored medium was obtained from Shanghai Comagal Microbial Technology Co., Ltd. Other routine chemical reagents were supplied by Merck Chemical Technology (Shanghai) Co., Ltd. or Sinopharm Chemical Reagent Co., Ltd. All enzymes and primers for DNA manipulation were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Two AHL biosensor strains, *Chromobacterium violaceum* CV026 [31] and *Agrobacterium tumefaciens* NTL4 (pZLR4) [32], were utilized for detecting AHLs with short and long acyl chains, respectively.

Sample Collection

The sampling site was located in the East China Sea near Zhoushan city (coordinates: 30° 14′ 43. 135″ N,122° 0′ 15.689″ E). The sediment samples were collected from a water depth of 16 m using a stainless-steel core sampler. The water conditions at the sampling site were as follows: temperature 20.3 °C, salinity 24.9 mg/L, pH 8.02, and dissolved oxygen (DO) content 7.93 mg/L. The sediment was placed into individual sterile plastic bags which were stored on ice and transported to the laboratory within 24 h of collection.

Screening of AHL-Producing *Vibrio*

Approximately 10.0 g of sediment was homogenized in 90 mL of sterile physiological saline for 1 min. The sample supernatant was serially diluted in sterile test tubes. 100 µl of bacterial suspension from each dilution was spread onto the surface of an LB agar plate and incubated at 28 °C for 72 h. Bacteria with observable differences in morphology were isolated and purified, and then the biosensor strains, *C. violaceum* CV026 and *A. tumefaciens* NTL4 (pZLR4), were used to further screen for AHL-producing bacteria. A full-grown culture (1 mL) of *C. violaceum* CV026 (a short-chain AHL biosensor strain) was incubated on LB agar medium supplemented with kanamycin (50 µg/mL) and the culture supernatant of isolated bacteria was added into the hole of LB agar plate, then incubating at 28 °C for 24 h. Similarly, the bacterial suspension was analyzed for long-chain AHL activity on an LB agar plate (gentamicin 30 µg/mL, X-gal 40 µg/mL) with *A. tumefaciens* NTL4 (pZLR4). Bacterial strains with a purple or blue chromatic reaction were further selected and inoculated onto CHROMagar *Vibrio* colored

medium and TCBS selective medium. Finally, AHL-producing *Vibrio* colonies were screened based on their morphological characteristics when cultured on *Vibrio* selective medium. The shape and external cell morphology were determined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

16S rRNA Sequencing and Phylogenetic Analysis

The physiological and biochemical characteristics of the *Vibrio* isolate were analyzed using the API 20 NE test system according to the manufacturer's instructions [33]. Antibiotic susceptibility testing was conducted using the disk diffusion method to identify growth inhibition zones. Genomic DNA was extracted using the QIAamp DNA Mini Kit. A polymerase chain reaction (PCR) was used to detect the bacterial 16S rRNA gene, performed using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'). The PCR cycling conditions were 95 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 2 min, with a final extension of 72 °C for 10 min. PCR product sequencing analysis was performed by Sangon Biotech Co., Ltd. (Shanghai, China). Result was compared to sequences within the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/) using BLASTn and a phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA) version 6.06 [34].

Growth and AHL Activity Experiments

The *Vibrio* isolate was grown for 16–20 h in LB broth at 28 °C, washed once with phosphate-buffered saline (PBS) and adjusted to $OD_{600} = 1.0$. Cells were inoculated into 20 mL LB broth (1% inoculum volume) and then cultured at 20 °C or 28 °C in a rotary shaker for 48 h at 160 rpm. The culture was sampled once every 6 h to measure the cell density (OD_{600}) and AHL activity. Determination of AHL activity was based on the blue circle diffusion assay. 100 µL of culture supernatant obtained after centrifugation (10,000 × *g*, 10 min) was added into the wells (φ7 mm) of an LB agar plate, which had been inoculated with *A.tumefaciens* NTL4 (pZLR4) and X-Gal reagent. The plates were then cultured at 28 °C for 24 h. Ultimately, the blue circle diameters were accurately measured using a caliper. Activity experiments were performed in triplicate.

Extraction and Detection of AHLs using UHPLC-MS/MS

The *Vibrio* isolate was inoculated into LB fluid medium and cultured at 28 °C with shaking at 160 rpm for 24 h. Cells were pelleted by centrifugation for 10 min at 10,000 × *g*. The supernatants were collected and used to extract AHLs for twice with an equal volume of acidified ethyl acetate (0.1% v/v glacial acetic acid) [35]. The organic phase containing AHLs was collected and evaporated using a rotary evaporator. The residue was dissolved in methanol (1.0 mL) and then filtered using a 0.22-µm Millipore membrane filter.

5 µl of the AHL extract was then analyzed by UHPLC-ESI–MS/MS using an Acquity UHPLC system (Waters Corp., Milford, MA, USA) coupled to a Xevo TQ-S triple-quadrupole tandem mass spectrometer [14]. Chromatographic separations were performed on a 1.7 µm × 2.1 mm × 100 mm Waters Acquity UHPLC BEH-C18 column maintained at 40 °C. The mobile phase was composed of A = methanol + 0.1% (v/v) acetic acid and B = ultrapure water + 0.1% (v/v) acetic acid. The gradient elution profile was as follows: 0–1 min, 60% A/40% B; 1–4.5 min, 95% A/5% B; 4.5–10 min, 60% A/40% B. The flow rate was 0.2 mL/min and the column eluent was directed to the Xevo TQ-S mass spectrometer using a multiple reaction monitoring system in a positive ion mode (ESI^+). Specific mass parameters were as follows: cone voltage, 36 V; capillary voltage, 2.5 kV; desolvation temperature, 500 °C; cone gas flow, 150 L/h; desolvation gas flow, 1000 L/h. A full scan with a mass range of m/z 50 to 500 Da was performed to confirm putative AHLs, as the fragment ion that was monitored at m/z 102 Da was based on a stable lactone ring.

16S rRNA Gene Sequence Accession Number

The 16S rRNA gene sequence for strain BJ-1 was deposited in GenBank database and assigned accession number OK161097.

Results

Isolation and General Features of AHL-Producing *Vibrio*

A total of nine AHL-producing bacteria were isolated from the sediment sample using two different reporter strains. Among them, a positive isolate, designated as BJ-1, could grow on the selective mediums, TCBS and CHROMagar, and exhibited typical yellow (Figure S1A) and violet (Figure S1B) colonies, respectively. Strain BJ-1 induced a coloration only in the biosensor *A. tumefaciens* NTL4 (pZLR4) (Figure S1D), indicating the production of medium and long-chain AHL molecules by BJ-1. Colonies of strain BJ-1 were round, translucent, smooth, and pale yellow after incubation on an LB agar plate at 28 °C for 24 h (Figure S1C). Gram staining was negative and no spores were observed. TEM (Fig. 1A) and SEM (Fig. 1B) images of BJ-1 showed long, rod-shaped

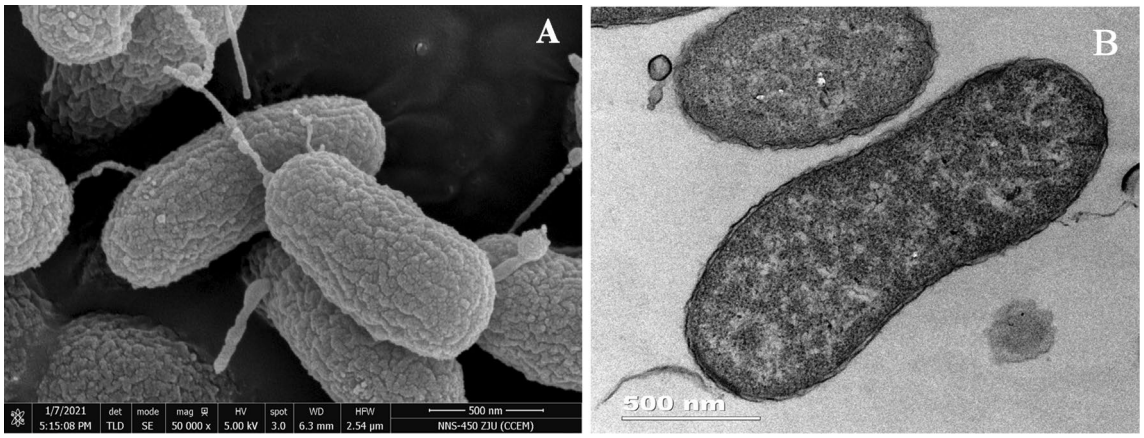


Fig. 1 Electron micrograph of *V. fluvialis* strain BJ-1. **A** Transmission electron micrograph, *Bar* 500 nm; **B** scanning electron micrograph, *Bar* 500 nm. Both TEM and SEM showed BJ-1 cells in long rods about 0.5–0.7 µm × 1.2–1.9 µm in size, with a single polar flagellum

cells of approximately 0.5–0.7 µm × 1.2–1.9 µm in size, with a single polar flagellum.

Identification of the Isolated Strain and Phylogenetic Analysis

The API 20NE identification system was employed to assess the physiological and biochemical characteristics of stain BJ-1. Using API 20NE, positive results were obtained for β-galactosidase, arginine dihydrolase, indole production, gelatinase, cytochrome oxidase, acid production from glucose, mannitol, sucrose, amygdalin, and arabinose. Negative results were obtained for lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophan deaminase, acetoin production (VP), oxidation/fermentation of inositol, sorbitol, rhamnose, and melibiose. All results were completely consistent with the biochemical characteristics of *V. fluvialis*. Importantly, there was no gas production from D-glucose by BJ-1, making it significantly different from *V. furnissii*, which does produce gas from D-glucose [36, 37]. Based on physiological and biochemical parameters, stain BJ-1 was preliminarily identified as *V. fluvialis*.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that the isolate BJ-1 clustered with *V. fluvialis* (Fig. 2) and possessed the highest similarity (99.77%) to *V. fluvialis* strain NCTC 11,327 and was also closely related to the *V. fluvialis* type strain NBRC 103,150 (99.44% sequence

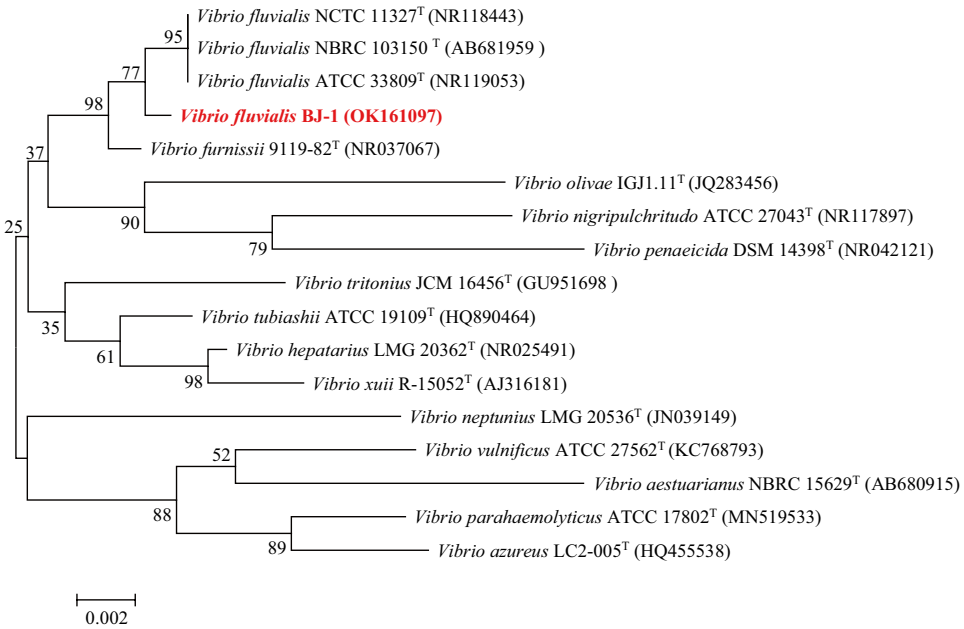


Fig. 2 Phylogenetic analysis of the AHL-producing stain BJ-1. The 16S rRNA-based phylogenetic tree was constructed using neighbor-joining and maximum-likelihood methods. Bootstrap values based on 1000 replicates are indicated at branch points. The scale bar 0.002 at the bottom represents evolutionary distance

similarity) and strain ATCC 33,809 (99.29% sequence similarity). Furthermore, the 16S rRNA gene sequence of strain BJ-1 showed 99.23% sequence similarity to *V. furnissii* strain 9119–82, identifying their close relationship, which is in concordance with the above results. Ultimately, the AHL-producing strain BJ-1 was identified as the zoonotic pathogen, *V. fluvialis*, according to phylogenetic analysis, as well as its morphological, physiological, and biochemical characteristics.

Bacterial Growth and AHL-Producing Activity

The growth and AHL-producing activity of BJ-1 were analyzed by measuring the OD_{600} values and diameters of the blue circle. As shown in Fig. 3, BJ-1 cells grew rapidly at 28 °C and reached the stationary phase after 12 h; the maximum cell density (OD_{600} = 2.79) was observed at 42 h. Maximum AHL activity was observed in the initial period (6 h) and decreased sharply after 18 h. At 20 °C, AHL activity firstly increased with increasing cell density until the cells were in stationary phase, and reached a maximum value at 18 h, before declining. As shown in Fig. 3, the maximum cell density differed little between the 28 °C and 20 °C culture conditions, whereas BJ-1 exhibited a slightly slower growth rate at 20 °C, indicating that a temperature of 28 °C was more suitable for rapid growth of strain BJ-1. Notably, BJ-1 showed high AHL-producing activity at low cell density, although this activity decreased when the cell density remained at a high level. Thus, a low cell density triggers *V. fluvialis* BJ-1 to increase the production of AHL signaling molecules that induces an increase in population density; conversely, BJ-1 reduces AHL signal secretion in response to high population density.

Identification of AHLs Profiling

UHPLC fractionation followed by ESI^+-MS/MS analysis of the AHL extracts was performed to identify AHL types. Two linear chain AHL products, C_8-HSL and C_{10}-HSL with retention times (RT) of 2.45 min and 2.74 min, were detected using a single-channel mode by precursor ion scan monitoring for m/z 228.1 and m/z 256.1, respectively (Fig. 4). The presence of three β-carbon-oxidative AHL products (3-oxo-C_8-HSL, 3-oxo-C_{10}-HSL, and 3-oxo-C_{12}-HSL) was also identified with respective RTs of 2.08, 2.46, and 2.75 min (Fig. 4). Referring to the total ion current chromatograms of the standards (Figure S2), the characteristic peak retention times of the five AHL products corresponded to those of the respective standard AHLs. In addition, MS/MS analysis of product peaks showed the product ions of m/z 228.0 → 102.0, m/z 256.0 → 102.1, m/z 242.0 → 102.1, m/z 270.1 → 102.0, and m/z 298.3 → 102.1, which were consistent with the ion forms of AHL synthetic standards (Fig. 5 and S3). Based on UHPLC and MS/MS analysis, the results confirmed that *V. fluvialis* BJ-1 produced five types of AHL, namely C_8-HSL, C_{10}-HSL, 3-oxo-C_8-HSL, 3-oxo-C_{10}-HSL, and 3-oxo-C_{12}-HSL.

Discussion

Vibrio spp. are a group of ubiquitous bacteria commonly found in marine and estuarine environments, of which a portion are pathogenic to many aquaculture organisms, and could also cause severe extra-intestinal infections in human. In some marine *Vibrio* species, QS systems are well studied and have been demonstrated to regulate the various physiological functions in response to hostile conditions, involving in bioluminescence [38], biofilm formation [39, 40], and virulence factor production [41]. The regulation of QS phenomenon is mediated by a typical group of small autoinducer molecules, such as AHLs, which have been detected in a total of 32 marine *Vibrio* species [20]. Ten short side-chain and 13 long side-chain AHLs have already been classified by using different detection methods; however, the AHL types and proportions largely differ among marine *Vibrio* [20].

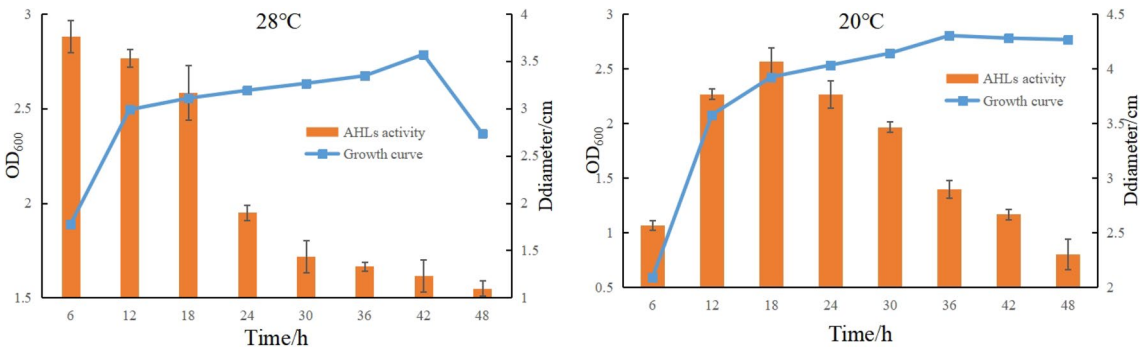


Fig. 3 Growth curve and AHLs production activity of stain BJ-1 under two different temperature conditions (28 °C and 20 °C). Error bars represent the standard deviations of three replicates

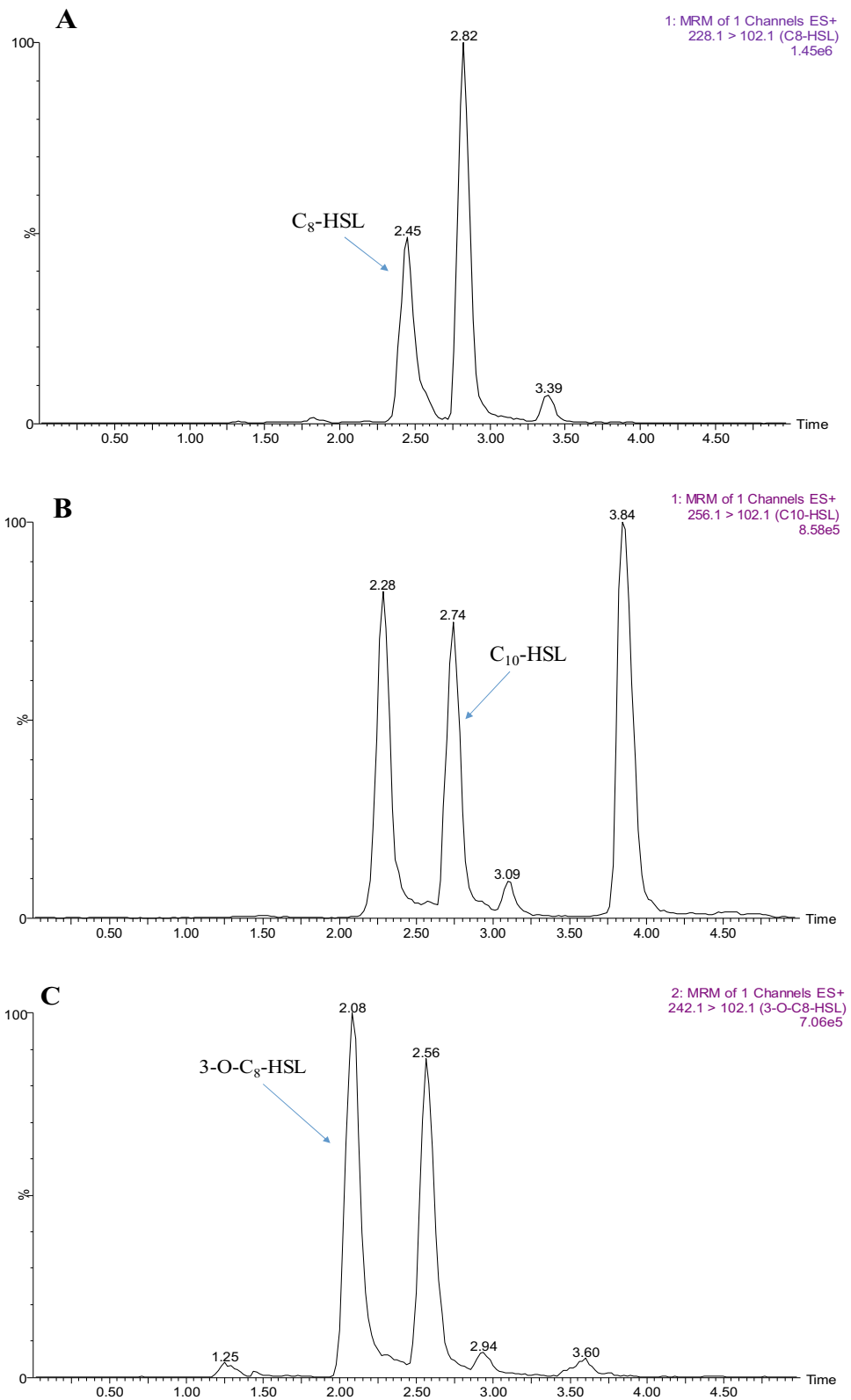
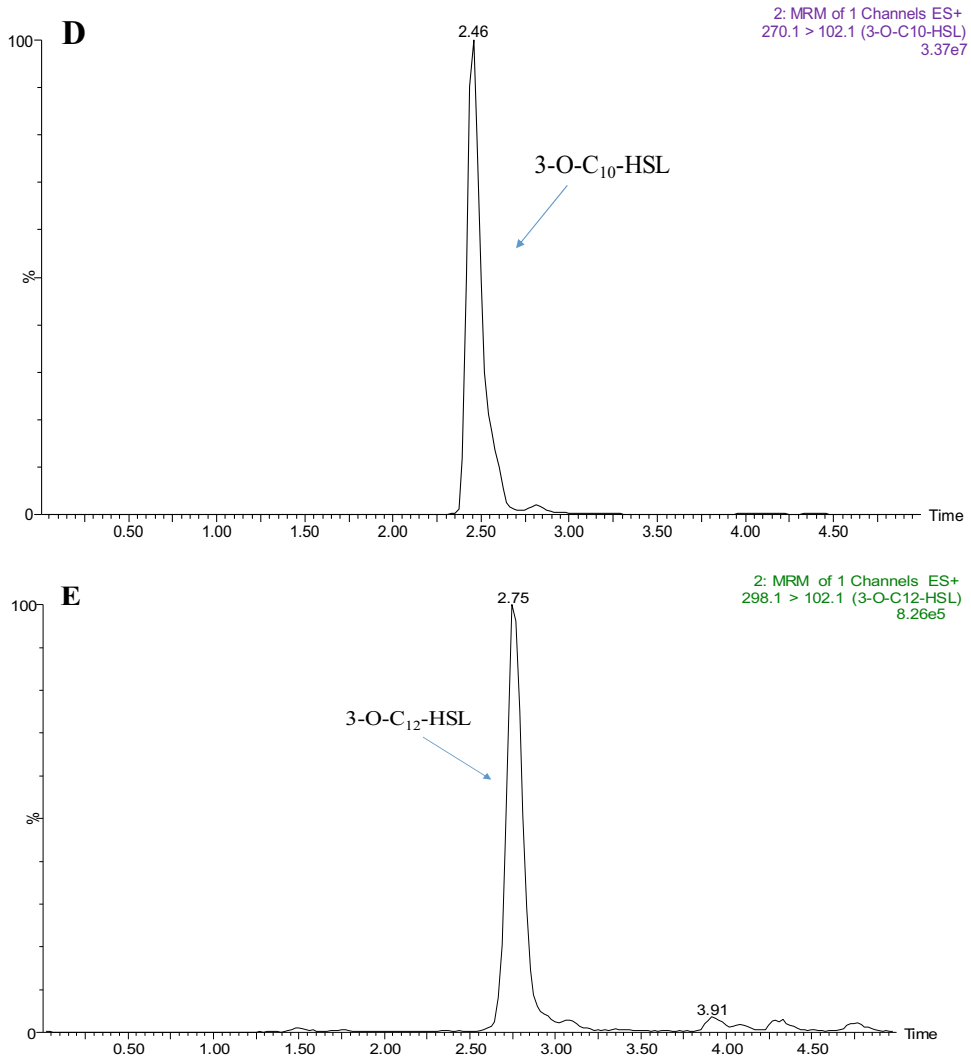


Fig. 4 Representative UHPLC-ESI–MS/MS chromatograms of the major AHL products extracted from *V. fluvialis* BJ-1 culture supernatant. Characteristic peaks with retention times of **A** 2.45 min for C_8-HSL, **B** 2.74 min for C_{10}-HSL, **C** 2.08 min for 3-oxo-C_8-HSL, **D** 2.46 min for 3-oxo-C_{10}-HSL, and **E** 2.75 min for 3-oxo-C_{12}-HSL

Fig. 4 (continued)



This pattern of AHL diversity may be closely associated with the survival and the pathogenicity of marine *Vibrio*. Thus, AHL types being accurately detected is considered as a critical link in QS-related studies in marine *Vibrio*.

The vast majority of AHL-producing *Vibrio* species had been well assessed for AHL types by physicochemical detection methods. Overall, 3-oxo-C_{10}-HSL, 3-oxo-C_{12}-HSL, 3-OH-C_4-HSL, and 3-OH-C_{12}-HSL are the most common AHL molecules in these *Vibrio* species [23]. Recently, an emerging pathogenic *Vibrio* species, named *V. fluvialis,* has received increasing public health concerns as it causes severe acute infections in human [26]. The QS regulatory pathways in *V. fluvialis* had been confirmed to play an important physiological role in pathogenesis, showing a positive regulation of the extracellular protease and hemolysin production [28]. The AHL-mediated QS represents one of the most studied QS system in *V. fluvialis*. Based on current literatures, a total of 11 AHLs produced by marine *V. fluvialis* had been definitely classified, and, however, it is clear that AHLs profiles were significantly different between various *V. fluvialis* strains [20].

Indeed, the AHL profiles are difficulty characterized since detection methods differ in their resolution and sensitivity. For example, Yang et al. [30] used the TLC assay with biosensor *A. tumefaciens* KYC55 to show that *V. fluvialis* VIB292 produced two signaling molecules, i.e., C_6-HSL and C_8-HSL. Based on a similar TLC-biosensor method, Wang et al. [28] subsequently demonstrated the presence of two AHL molecules (C_{10}-HSL and 3-oxo-C_{10}-HSL) in *V. fluvialis* culture supernatants. However, three AHL molecules, i.e., C_{10}-HSL, 3-oxo-C_{10}-HSL, and 3-oxo-C_{12}-HSL, were detected using ESI–MS/MS analysis. The ESI–MS/MS results showed that 3-oxo-C_{10}-HSL was the major AHL component, and that C_{10}-HSL and 3-oxo-C_{12}-HSL were the minor components [28]. These comparative data suggest that ESI–MS/MS has a higher sensitivity and specificity for AHL detection in *V. fluvialis*. Lately, Rasmussen et al. [29] used UHPLC-DAD-QTOFMS to show that *V. fluvialis* S1162 can produce multiple

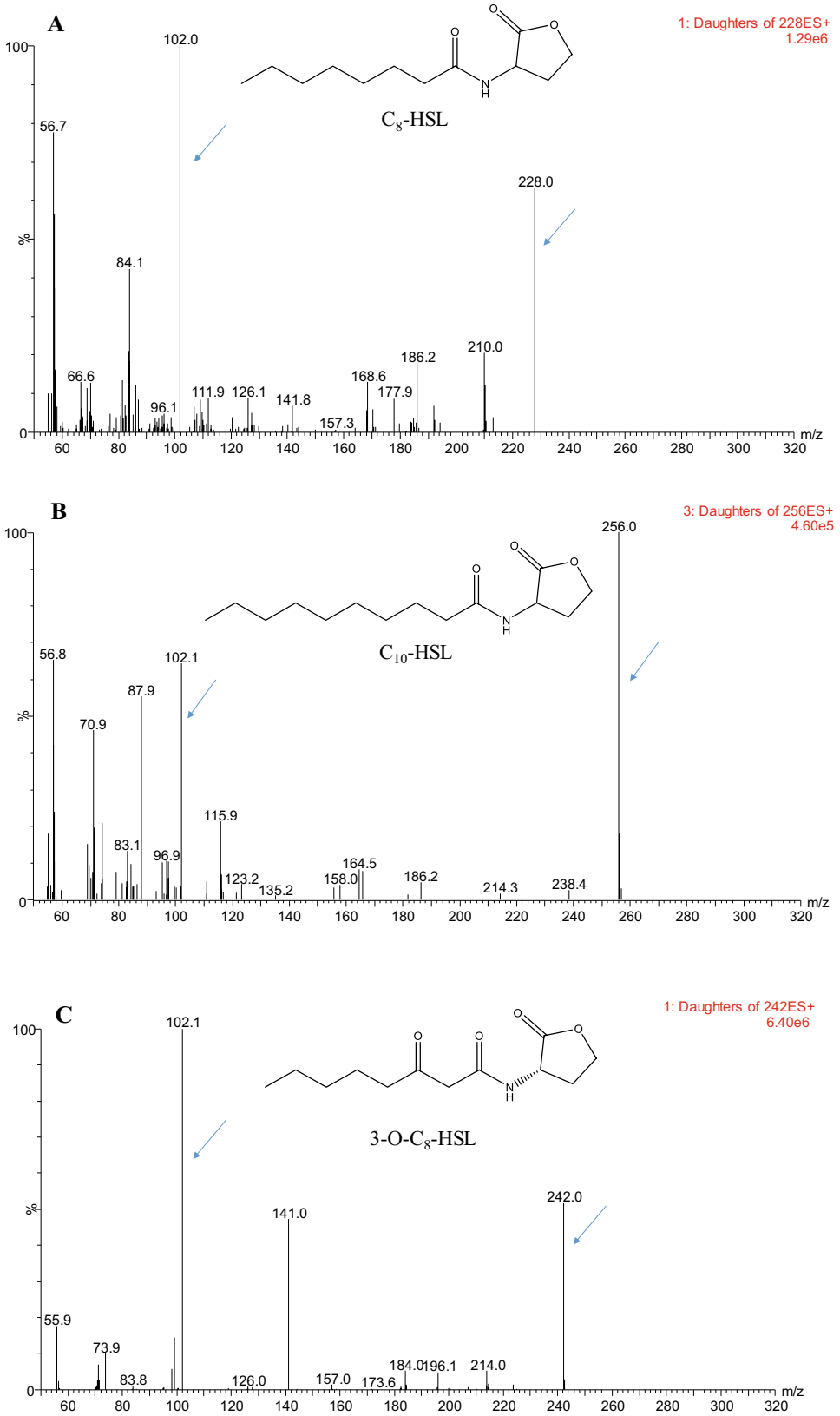
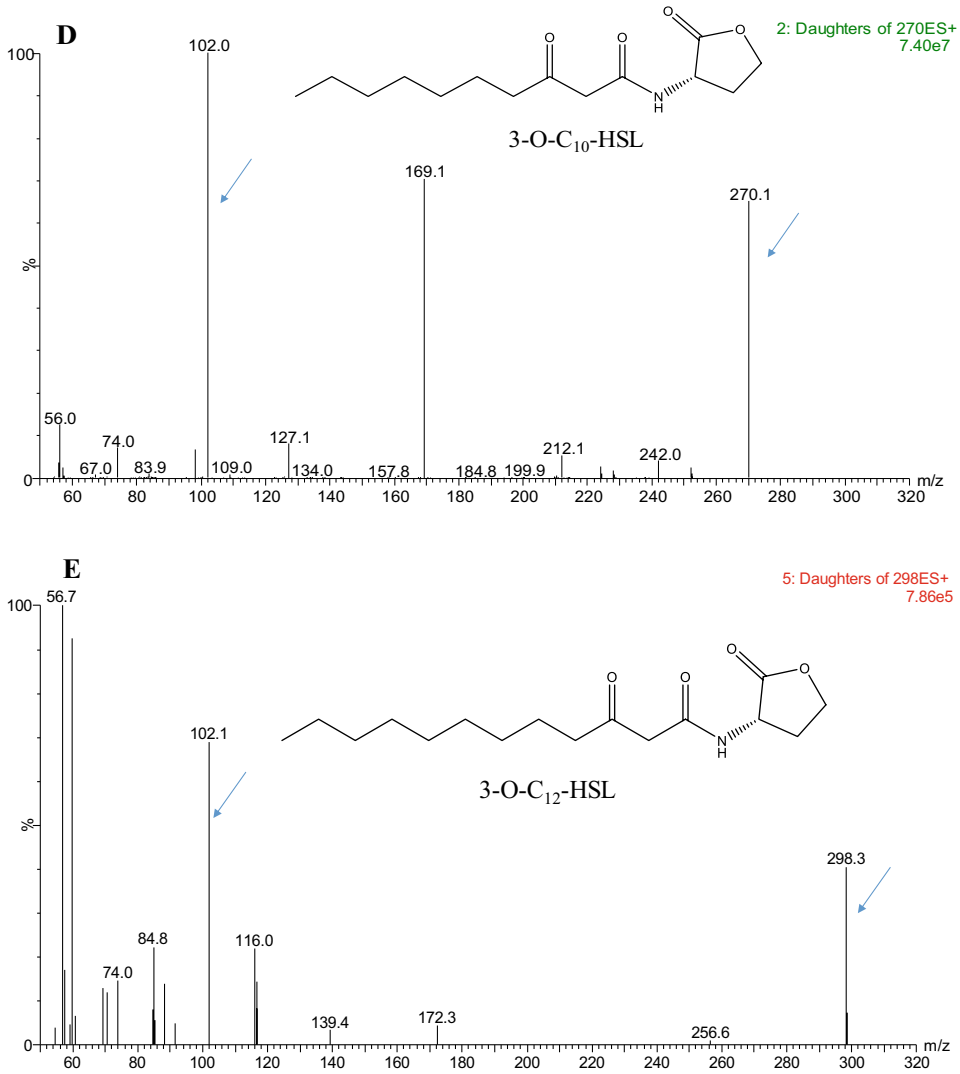


Fig. 5 Mass spectra of five AHL products extracted from the chromatographic peaks at their respective retention times. The major ion peaks for respective **A** C_8-HSL (*m/z* 228.0 → 102.0), **B** C_{10}-HSL (*m/z* 256.0 → 102.1), **C** 3-O-C_8-HSL (*m/z* 242.0 → 102.1), **D** 3-O-C_{10}-HSL (*m/z* 270.1 → 102.0), and **E** 3-O-C_{12}-HSL (*m/z* 298.3 → 102.1) are marked by arrows

Fig. 5 (continued)



AHL molecules, including two β-carbon-hydroxylated AHLs and six β-carbon-oxidative AHLs. Nevertheless, it is difficult to verify the accuracy of these findings due to the low response value of partial confirmed AHL products as well as a failure to detect linear chain AHLs. In the present study, five AHLs were successively isolated via the ACQUITY UHPLC I-Class system and their molecular structures were accurately determined by ESI–MS/MS. As a result, the coupling of UHPLC to ESI–MS/MS allowed each AHL product to be correctly identified and quantified based on retention time, molecular mass, as well as MS/MS fragmentation and comparison to the standard AHLs, resulting in a more reliable analysis. In future, to meet the demands for rapid and accurate AHL determination, it is believed that the UHPLC-ESI–MS/MS method will be great application value and considerable potential for marine *Vibrio* AHL detection and analysis.

Conclusion

An AHL-producing bacterium, *V. fluvialis* BJ-1, was isolated from marine sediments using the biosensor *A. tumefaciens* NTL4 (pZLR4). Morphological, physiological, and biochemical characteristics and phylogenetic analyses classified it as the marine opportunistic pathogen, *V. fluvialis*. Although bacterial growth curves showed that the maximum cell densities when cultured under two temperature conditions were similar, a temperature of 28 °C was considered more suitable for strain BJ-1 growth in view of the slightly faster growth rate. The results of the AHL activity experiment revealed that the AHL signaling molecules produced by *V. fluvialis* BJ-1 had a negative regulatory effect on bacterial population density. AHLs extracted from the culture supernatants of *V. fluvialis* BJ-1 were detected by UHPLC-MS/MS. Based on comparative chromatography-mass spectrometry data with AHL

synthetic standards, five different AHL molecules were accurately identified as C_8-HSL,C_{10}-HSL,3-oxo-C_8 -HSL, 3-oxo-C_{10}-HSL, and 3-oxo-C_{12}-HSL.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-022-02879-5.

Acknowledgements Not applicable.

Author Contributions The authors' contribution to this manuscript is as follows: Jin conceptualized and designed the study. Bao prepared materials, collected data and carried out chromatographic analysis. Guo wrote the first draft of the manuscript. Li and Shi provided the resources and significant input on the data interpretation. All authors critically revised all versions of the manuscript, and approved the final manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or nonprofit sectors.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Consent for Publication Not applicable.

Research Involving Human Participants and/or Animals This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Waters CM, Bassler BL (2005) Quorum sensing: Cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346
2. Schuster M, Sexton DJ, Diggle SP, Greenberg EP (2013) Acyl-homoserine lactone quorum sensing: from evolution to application. Annu Rev Microbiol 67:43–63
3. Prescott RD, Decho AW (2020) Flexibility and adaptability of quorum sensing in nature. Trends Microbiol 28(6):436–444
4. Parker CT, Sperandio V (2009) Cell-to-cell signaling during pathogenesis. Cell Microbiol 11:363–369
5. Bandara HM, Lam OL, Jin LJ, Samaranayake L (2012) Microbial chemical signaling: a current perspective. Crit Rev Microbiol 38(3):217–249
6. Papenfort K, Bassler BL (2016) Quorum sensing signal-response systems in gram-negative bacteria. Nat Rev Microbiol 14:576–588
7. Fuqua C, Greenberg EP (2002) Listening in on bacteria: acyl-homoserine lactone signalling. Nat Rev Mol Cell Biol 3(9):685–695
8. Parsek MR, Greenberg EP (2000) Acyl-homoserine lactone quorum sensing in gram-negative bacteria: A signaling mechanism involved in associations with higher organisms. Proc Natl Acad Sci USA 97:8789–8793
9. Jin L, Bao J, Chen Y, Yang W, Du W (2021) Structural insights into acyl-ACP selective recognition by the *Aeromonas hydrophila* AHL synthase AhyI. BMC Microbiol 21(1):1–11
10. Reading NC, Vanessa S (2006) Quorum sensing: the many languages of bacteria. FEMS Microbiol Lett 254:1–11
11. O'Connor G, Knecht LD, Salgado N, Strobel S, Pasini P, Daunert S (2015) Whole-cell biosensors as tools for the detection of quorum-sensing molecules: uses in diagnostics and the investigation of the quorum-sensing mechanism. Adv Biochem Eng Biotechnol 3:181–200
12. Sun L, Tang C, Leng YP (2010) The research progress of the novel thin layer chromatography expansion agent. Sci Technol West China 9:47–48
13. Okutsu N, Morohoshi T, Xie X, Kato N, Ikeda T (2016) Characterization of N-acylhomoserine lactones produced by bacteria isolated from industrial cooling water systems. Sensors 16(1):44
14. Jin L, Zhang X, Shi H, Wang W, Qiao Z, Yang W, Du W (2020) Identification of a novel N-acyl Homoserine lactone synthase, AhyI, in *Aeromonas hydrophila* and structural basis for its substrate specificity. J Agr Food Chem 68(8):2516–2527
15. Jin L, Chen Y, Yang W, Qiao Z, Zhang X (2020) Complete genome sequence of fish-pathogenic *Aeromonas hydrophila* HX-3 and a comparative analysis: insights into virulence factors and quorum sensing. Sci Rep 10(1):1–15
16. Klitgaard A, Iversen A, Andersen MR, Larsen TO, Frisvad JC, Nielsen KF (2014) Aggressive dereplication using UHPLC-DAD-QTOF: screening extracts for up to 3000 fungal secondary metabolites. Anal Bioanal Chem 406(7):1933–1943
17. Taylor MW, Schupp PJ, Baillie HJ, Charlton TS, de Nys R, Kjelleberg S, Steinberg D, P, (2004) Evidence for acyl homoserine lactone signal production in bacteria associated with marine sponges. Appl Environ Microbiol 70:4387–4389
18. Ferreira NP, Chiavelli LUR, Lucca DL, de Oliveira Santin SM, Pavli F, Nychas GJ, Zuluagac MYA, de Oliveira ALM, Pomini AM (2019) Identification and characterization of a long-chain N-acyl homoserine lactone from *Rhizobium* sp. isolated from Zea x mays rhizosphere. Rhizophere-Neth 9:34–37
19. Girard L, Blanchet É, Intertaglia L, Baudart J, Stien D, Suzuki M, Lebaron P, Lami R (2017) Characterization of N-Acyl homoserine lactones in *Vibrio tasmaniensis* LGP32 by a biosensor-based UHPLC-HRMS/MS Method. Sensors 17:906
20. Liu J, Fu K, Wu C, Qin K, Li F, Zhou L (2018) In-Group communication in marine vibrio a review of N-acyl homoserine lactones-driven quorum sensing. Front Cell Infect Mi 8:139
21. Milton DL (2006) Quorum sensing in *vibrios*: complexity for diversification. Int J Med Microbiol 296(2–3):61–71
22. Pruzzo C, Huq A, Colwell RR, Donelli G (2005) Pathogenic *Vibrio* species in the marine and estuarine environment. In: Oceans and health: pathogens in the marine environment. Springer Boston, MA p: 217–252.
23. Girard L (2019) Quorum sensing in Vibrio spp. the complexity of multiple signalling molecules in marine and aquatic environments. Crit Rev Microbiol 45(4):451–471
24. Dunlap PV (1999) Quorum regulation of luminescence in *Vibrio fischeri*. J Mol Microbiol Biotechnol 1(1):5–12
25. Tall BD, Fall S, Pereira MR, Ramos-Valle M, Curtis SK, Kothary MH, Chu DMT, Monday SR, Kornegay L, Donkar T, Prince D, Thunberg RL, Shangraw KA, Hanes DE, Khambaty FM, Lampel KA, Bier JW, Bayer RC (2003) Characterization of *Vibrio fluvialis*-like strains implicated in limp lobster disease. Appl Environ Microbiol 69(12):7435–7446
26. Ramamurthy T, Chowdhury G, Pazhani GP, Shinoda S (2014) *Vibrio fluvialis*: an emerging human pathogen. Front Microbiol 5:91
27. Zheng B, Jiang X, Cheng H, Guo L, Zhang J, Xu H, Yu X, Huang C, Ji J, Ying CQ, Feng YJ, Xiao YH, Li L (2017) Genome characterization of two bile-isolated *Vibrio fluvialis* strains: an insight into pathogenicity and bile salt adaption. Sci Rep 7(1):1–10

28. Wang Y, Wang H, Liang W, Hay AJ, Zhong Z, Kan B, Zhu J (2013) Quorum sensing regulatory cascades control *Vibrio fluvialis* pathogenesis. J Bacteriol 195(16):3583–3589
29. Rasmussen BB, Nielsen KF, Machado H, Melchiorsen J, Gram L, Sonnenschein EC (2014) Global and phylogenetic distribution of quorum sensing signals, acyl homoserine lactones, in the family of *Vibrionaceae*. Mar Drugs 12(11):5527–5546
30. Yang Q, Han Y, Zhang XH (2011) Detection of quorum sensing signal molecules in the family *Vibrionaceae*. J Appl Microbiol 110(6):1438–1448
31. McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, Daykin M, Lamb JH, Swift S, Bycroft BW, Stewart GSAB, Williams P (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiol Spectr 143(12):3703–3711
32. Zhu J, Chai Y, Zhong Z, Li S, Winans SC (2003) *Agrobacterium* bioassay strain for ultrasensitive detection of N-acylhomoserine lactone-type quorum-sensing molecules: detection of autoinducers in *Mesorhizobium huakuii*. Appl Environ Microbiol 69(11):6949–6953
33. Bosshard PP, Zbinden R, Abels S, Boddinghaus B, Altwegg M, Bottger EC (2006) 16S rRNA gene sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of nonfermenting Gram-negative bacteria in the clinical laboratory. J Clin Microbiol 44(4):1359–1366
34. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30(12):2725–2729
35. Ravn L, Christensen AB, Molin S, Givskov M, Gram L (2001) Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL-production kinetics. J Microbiol Methods 44(3):239–251
36. Brenner DJ, Hickman-Brenner FW, Lee JV, Steigerwalt AG, Fanning GR, Hollis DG, Farmer JJ, Weaver RE, Joseph SW, Seidler RJ (1983) *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. J Clin Microbiol 18(4):816–824
37. Lu X, Liang W, Wang Y, Xu J, Zhu J, Kan B (2014) Identification of genetic bases of *Vibrio fluvialis* species-specific biochemical pathways and potential virulence factors by comparative genomic analysis. Appl Environ Microbiol 80(6):2029–2037
38. Miyashiro T, Ruby EG (2012) Shedding light on bioluminescence regulation in *Vibrio fischeri*. Mol Microbiol 84(5):795–806
39. Zhou D, Yan X, Qu F, Wang L, Zhang Y, Hou J, Hu Y, Li J, Xin S, Qiu J, Yang R, Mao P (2013) Quorum sensing modulates transcription of cpsQ-mfpABC and mfpABC in *Vibrio parahaemolyticus*. Int J Food Microbiol 166(3):458–463
40. García-Aljaro C, Melado-Rovira S, Milton DL, Blanch AR (2012) Quorum-sensing regulates biofilm formation in *Vibrio scophthalmi*. BMC Microbiol 12(1):1–9
41. Higgins DA, Pomianek ME, Kraml CM, Taylor RK, Semmelhack MF, Bassler BL (2007) The major *Vibrio cholerae* autoinducer and its role in virulence factor production. Nature 450(7171):883–886