



Quorum Quenching Enzyme APTM01, an Acylhomoserine-Lactone Acylase from Marine Bacterium of *Pseudoalteromonas tetraodonis* Strain MQS005

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Abstract

Quorum sensing is a system of stimuli and response correlated to population density and involves in pathogen infection, colonization, and pathogenesis. Quorum quenching enzymes as quorum sensing inhibitors have been identified in a number of bacteria and been used to control by triggering the pathogenic phenotype. The marine bacteria of *Pseudoalteromonas* had wide activity of degrading AHLs as a type of signal molecule associated with quorum sensing. We screened many *Pseudoalteromonas* strains in large scale to explore genes of quorum quenching enzymes from the China seas by whole-genome sequencing rather than genomic library construction. Nine target strains were obtained and an acylases gene APTM01 from the strain MQS005 belonging to PvdQ type on sub-branch in phylogenetic tree. And the heterogenous host containing the vector with target gene could degrade C10-HSL, C12-HSL and OC12-HSL. The obtained AHL acylase gene would be a candidate quorum quenching gene to apply in some fields. We identified that the strains of *Pseudoalteromonas* have wide AHL-degrading ability depending on quorum quenching. The strains would be a resource to explore new quorum quenching enzymes.

Introduction

Quorum sensing (QS) is a signaling system that occurs in the bacterial population to sense population density and synchronize the expression of specific gene via the secretion of small, diffusible signal molecules [1]. QS was first discovered in a luminescent marine bacterium of *Vibrio Fay* (*Vibrio fischeri*) [2]. Bacteria exchange the chemical signals between cells by secreting substance one or

more “autoinducer (AI)” to extracellular environment [3]. QS could regulate 10–30% genes’ expression in bacterial genomes [4–6], including secondary metabolites of bacteria, biofilm formation, pathogenic factor expression, cell movement and a series of important functional genes [7]. Different bacteria usually contain different signal molecules and different regulation mechanisms. The compound of acylhomoserine-lactone (*N*-acyl-homoserine lactone, AHL) in Lux QS system of Gram-negative bacteria is the most commonly studied QS substance [8]. Researches revealed that the QS is the key component of respiratory diseases caused by *Pseudomonas aeruginosa*. The QS system in *P. aeruginosa* controls the bacterial adhesion, biofilm formation and expression of virulence factors [9]. *Burkholderia glumae* which caused bacterial blight pathogen of rice synthesizes *N*-octanoyl-L-homoserine lactone. But it could not product flagella to aggregate and the pathogenicity of rice was lost though it can produce toxoflavin, when the gene was mutated [10]. These results showed that bacterial QS systems regulated by AHLs play a key role to control bacterial disease, involving in pathogen infection, colonization, and pathogenesis.

Many quorum quenchers including analogs and degrading enzymes were explored for inhibiting specific gene expression

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of pathogens, which has become an important research direction [11]. Quorum quenching enzymes belonging to degrading enzymes had been identified in a number of bacteria that have shown considerable promise as quorum quenchers. These enzymes have been identified in many microbes, including lactonase [12], acylase [13], oxidoreductase [14] and paraoxonase [15, 16]. Lactonase hydrolyzes the ester bond of the homoserine lactone ring of acylated homoserine lactones, and acylase hydrolyzes the acyl skeleton. AHL-lactonase AiiA belonging to the metallo- β -lactamase superfamily was first identified from *Bacillus* sp. strain 240B1 to attenuate virulence in *Erwinia carotovora* [17] which is a plant pathogen that causes soft rot in a number of crops such as potatoes and carrots [18] by using *N*-hexanoyl-L-homoserine lactone (C6-HSL) quorum sensing [19]. And plants expressing AHL-lactonase were shown to demonstrate enhanced resistance to infection from the pathogen *Erwinia carotovora* [20]. Many AiiA-like enzymes including AidC from *Chryseobacterium* sp. strain StRB126 [21], AiiM from *Microbacterium testaceum* StLB037 [12], AhID from *Arthrobacter* sp. strain IBN110 [22], AttM from *Agrobacterium tumefaciens* strains A6 and C58 [23] and QlcA from unculturable soil bacteria [24] were cloned. In *P. aeruginosa*, the AHL acylase PvdQ has been identified as a late responder to the 3-oxo-C12-HSL QS circuit [13]. And another quiP gene encoding acylase was also identified in *P. aeruginosa*, but it was not required for AHL utilization in the identical strain [25]. In the public databases of protein conserved domain, these two type AHL acylases contain an annotated pattern which could help to identify PvdQ and quiP genes according to protein sequences.

Pseudoalteromonas is a genus of marine bacterium and many studies reported that some strains of *Pseudoalteromonas* have extensive and significant anti-biofilm activity [26–29]. The product of violacein in *Pseudoalteromonas* bacteria has been identified [27, 30, 31], which is depending on QS system. Therefore, we speculate that AHL-degrading ability may be related to quorum quenching. Then we try to explore the strains of *Pseudoalteromonas* with AHL-degrading ability by screening marine bacteria in China seas. Nine target strains were obtained and an acylases gene APTM01 belonging to PvdQ type was found by whole-genome sequencing from *Pseudoalteromonas tetraodonis* strain MQS005 isolated from the water of South China Sea. To find high homology of AHL-degrading enzyme gene, this study provides new methods and ideas and is significant to the study of the functional domain of the enzyme.

Materials and Methods

Bacterial Strain Growth and Function Selection

Forty strains of *Pseudoalteromonas* were isolated from the water of South China Sea and were used to screen strains that had the potential function of degrading AHL(s). These strains were grown in the culture medium of autoclaved 2216E liquid medium (each liter contains 5 g peptone, 1 g yeast extract, 0.01 g ferric phosphate and 35 g conate sea salt with pH 7.6–7.8). Total of 7 AHLs including C6-HSL, C8-HSL, *N*-decanoyl-L-homoserine lactone (C10-HSL), *N*-dodecanoyl-L-homoserine lactone (C12-HSL), *N*-(3-oxooctanoyl)-L-homoserine lactone (OC8-HSL), *N*-(3-oxodecanoyl)-L-homoserine lactone (OC10-HSL), and *N*-(3-oxododecanoyl)-L-homoserine lactone (OC12-HSL), were synthesized by Sigma-Aldrich Co., LLC. (Table S1). The AHLs powder was dissolved in the mixture of methyl alcohol and ethyl acetate (v:v = 1:1). The culture medium of autoclaved 2216E liquid medium with different AHL solutions was used to culture each *Pseudoalteromonas* strains at 30 °C for 16 h. The cultured medium was centrifuged at 13,000 \times g for 15 min to remove the bacterial cells. Next, the mixture containing 400 μ L supernatant from the last step and 2 mL fresh LB medium (each liter contains 10 g tryptone, 5 g yeast extract, and 10 g NaCl with pH 7.2) was used to inoculate the reporter strains of *Chromobacterium violaceum* CV026 (ATCC 31532) and VIR07 (ATCC 12472), respectively. The inoculated mixture medium was with 200 rpm at 37 °C for 16 h. The thallus in the medium would not change to purple if the strain of *Pseudoalteromonas* could degrade the corresponding AHL(s), because the reporter strains CV026 and VIR07 responding to short-chain and long-chain AHLs, respectively show purple color when AHL exists.

Genomic DNA Extraction and Genome Sequencing

The candidate bacterial strains which could degrade AHLs were cultured in fresh 2216E liquid medium with 200 rpm at 30 °C for 16 h. Their genomic DNAs were extracted using TIANamp Bacteria DNA Kit (catalog NO. DP302, TIANGEN Biotech Co., Ltd., Beijing, China) and all operation was processed according to the protocol of the kit. Paired-end libraries with the insert size of 350 bp were constructed to sequence genome DNA. The libraries were sequenced on the Illumina HiSeq 2500 platform with PE150 strategy. Quality control of paired-end reads was performed using in-house program to filter Illumina PCR adapter and low-quality reads. The filtered reads were

assembled by SOAPdenovo [32, 33] to generate scaffolds and all reads were used for further gap closure.

Genome Sequence Annotation and Target Gene Screen

With gene prediction, the genome assembly of candidate bacterial strain was explored by GeneMarkS [34] (<http://topaz.gatech.edu/>) with integrated model and Heuristic model parameters. A whole-genome BLAST [35] search (<http://blast.ncbi.nlm.nih.gov/>) (E value $< 1e-5$, minimal alignment length percentage larger than 40%) was performed against four databases including Kyoto encyclopedia of genes and genomes (KEGG) [36], clusters of orthologous groups (COG) [37], nonredundant protein (NR) databases, and gene ontology (GO) [38], respectively. To compare the genome sequences of the same species, Mauve software [39] was employed to reorder the contigs/scaffolds of *P. tetraodonis* strain MQS005, and to align the reference draft genomes of *P. tetraodonis* including strain UCD-SED8 [40] and CSB01KR (https://www.ncbi.nlm.nih.gov/genome/40223?genome_assembly_id=281916), respectively. Then, all classified genes were screened to find the genes relative to degradation of AHLs, especially the genes of AHL acylase and acyl-homoserine lactonase. The sequences of candidate genes were aligned to the gene database with BLAST search again to determine the previous similar species and their similarity. And Top 5 similar AHL acylase sequences and the protein sequence of candidate gene were multiple-aligned by ClustalX [41], then the aligned results were imported to the software of GeneDoc [42] to analyze their conserved region.

Gene Cloning and Heterogenous Expression

The coding region of the candidate gene was amplified using chromosomal DNAs of candidate strains as a template and the oligonucleotide primers PF (5'-GGAATTCCATATGTTTATGTTTAAAGTAATCAAACGGTTGCTA-3') and PR (5'-CGGGATCCCTAGTCGTTTCATTGGTGCAATTC A-3'). The PCR conditions involved denature at 94 °C for 5 min followed by 27 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min with PrimeSTAR[®] Max DNA Polymerase (Code No. R045A, TAKARA Biotechnology Co., Ltd. Dalian, China). The PCR products were digested by *Nde*I and *Bam*HI and purified with E.Z.N.A Gel Extraction Kit (product no. D2500-01, Omega Bio-tek Inc.). The purified DNAs were ligated to the *Nde*I-*Bam*HI-digested pET15b vector and introduced into *Escherichia coli* DH5 α . Total of 20 transformants were selected and sequenced to obtain

target one with correct target gene fragment. The resulting expression vector was named pET15b-APT01.

Function Verification of Target Genes

Escherichia coli DH5 α containing pET15b-APT01 was cultured in LB medium with shaking of 200 rpm at 37 °C for 8 h. The expression vector was extracted with Plasmid Mini Kit I (product no. D6942-01, Omega Bio-tek Inc.) and introduced into the expressing host of *E. coli* BL21. *E. coli* BL21 with pET15b-APT01 was cultured in sterilized liquid LB medium containing AHLs at 37 °C until an OD₆₀₀ of 0.6 was achieved. Then, IPTG was added into the culture media at the final concentration of 1 mM for 16 h to induce the expression of the target gene. The cultured medium was centrifuged at 13,000 $\times g$ for 15 min to remove bacterial cells. AHL reporter strains of CV026 and VIR07 were employed to test the degradation of AHLs as described above. To explore the AHL-degrading activity of the target gene, a method of AHLs-diffusion on reporter plate with punched solid medium was used [12]. On the reporter plate, the reporter strains of CV026 and VIR07 was coated evenly on the plate. A hole with the diameter of 8 mm was punched and 100 μ L supernatant without cells was dripped into the hole carefully. The reporter plate was incubated at 37 °C for 16 h to measure the diameter of diffusion area. The all bacterial strains and plasmid used in this study were listed in Table 1.

Nucleotide Sequence Accession Number

The genome sequence of *P. tetraodonis* strain MQS005 was deposited at DDBJ/EMBL/GenBank under the accession no. LYRQ00000000. The version described in this paper is version LYRQ01000000.

Results

Bacterial Function Selection

Bacterial strains of *Pseudoalteromonas* were isolated from marine and their functions of degrading AHLs were tested. Total of 9 strains have different AHL-degrading activity, respectively (Table 2), but they could not produce AHLs without other inducers. The results also showed that all strains could not degrade OC8-HSL and OC10-HSL, and only the strain of NQ24 degraded C6-HSL slightly (Fig. S1). When testing their ability to degrade C8-HSL, it is obvious

Table 1 Bacterial strains and plasmids used in this study

| Strains or plasmids | Description | Source |
|----------------------------------|--|------------|
| Strains | | |
| <i>Pseudoalteromonas</i> sp. | AHL-degrading strain isolated from marine | This study |
| <i>Chromobacterium violaceum</i> | Signal molecule report strain | |
| CV026 | ATCC 31532 derivative, <i>cviI</i> ::Tn5xylE Km ^r , Sm ^r | |
| VIR07 | ATCC 12472 derivative, <i>cviI</i> ::Km ^r , Ap ^r | |
| Competent cell | | |
| <i>E. coli</i> DH5 α | F ⁻ , ϕ 80, <i>lacZ</i> Δ M15, Δ (<i>lacZYA-argF</i>) U169 end A1, <i>recA1</i> , <i>hsdR17</i> (r _K ⁻ , m _K ⁺) <i>supE44</i> , λ ⁻ , <i>thi-1</i> , <i>gyrA96</i> , <i>relA1</i> , <i>phoA</i> | TIANGEN |
| <i>E. coli</i> BL21(DE3) | F ⁻ , <i>ompT</i> , <i>hsdS_B</i> (r _B ⁻ , m _B ⁻), <i>gal</i> , <i>dcm</i> (DE3) | TIANGEN |
| Plasmids | | |
| pET15b | Expression vector; Ap ^r | Novagen |
| pET15b-APTM01 | pET15b vector containing putative AHL-degrading gene from MQS005 | This study |

Table 2 AHL-degrading ability of *Pseudoalteromonas* strains

| Name | C6-HSL | C8-HSL | OC8-HSL | C10-HSL | OC10-HSL | C12-HSL | OC12-HSL |
|--------|--------|--------|---------|---------|----------|---------|----------|
| HK5 | - | ++ | - | + | - | + | +- |
| MSQ004 | - | ++ | - | ++ | - | ++ | ++ |
| MQS005 | - | ++ | - | ++ | - | ++ | ++ |
| NQ24 | + | + | - | ++ | - | ++ | +- |
| BM7 | - | ++ | - | ++ | - | + | - |
| D5204 | - | ++ | - | ++ | - | ++ | ++ |
| D2139 | - | ++ | - | + | - | ++ | +- |
| D4003 | - | + | - | + | - | ++ | ++ |
| D2205 | - | + | - | ++ | - | + | + |

++, strong degradation; +, weak degradation; +-, weaker degradation; -, no degradation

that the tube colors of the strains HK5, NQ24 and D2139 were darker than the control in the reporting of VR07, and the tubes did not show any purple in the reporting of CV026, which indicated that they might secrete more other AHLs with longer-chain. And the strain of MQS005 was shown the similar result when testing its ability to degrade C6-HSL. Therefore, the strains of MQS005 were selected and sequenced to obtain their draft genome sequences.

Genome Draft Sequencing and Annotation

About the draft genome sequence of *P. tetraodonis* strain MQS005, a total of 1214 Mb of high-quality sequence data were obtained, and the resulting assembly consisted of 4252,538 bp containing 144 contigs (longest, 221,568 bp; N_{50} , 60,836 bp), with a G/C content of 41.85%. With gene prediction, up to 3943 genes in the genome assembly of *P. tetraodonis* strain MQS005 were found by GeneMarkS with integrated model and Heuristic model parameters. A total of 3738, 2222, 1977, and 2692 functional genes were annotated from KEGG, COG, NR, and GO, respectively.

We tried to find similar genomes of the same species from public databases. At present, only two genome sequences of *P. tetraodonis* strain (strain UCD-SED8 and CSB01KR) are available in the public database. Then, the alignments of the draft genome of MQS005 to the genome sequences of *P. tetraodonis* strain UCD-SED8 and CSB01KR were processed by Mauve program. Before aligning the draft genome of MQS005 to the published genomes, the genome sequences were reordered because these two genome sequences were draft sequences containing many contigs or scaffolds. Comparing to UCD-SED8, there were four fragment translocations including two reverses (Fig. 1a). Comparing to CSB01KR, there were four obvious fragment extensions, 13 fragment translocations and nine reverses (Fig. 1b). The alignment results showed that the genome sequences of *P. tetraodonis* strain MQS005 were much more similar to the strain CSB01KR though some fragments were not collinear to each other (Fig. 1). These two draft genome sequences were the only assemblies of *P. tetraodonis* in the public database, but the obtained target gene was not in their genome. Then, we screened all predicted/annotated genes to find functional

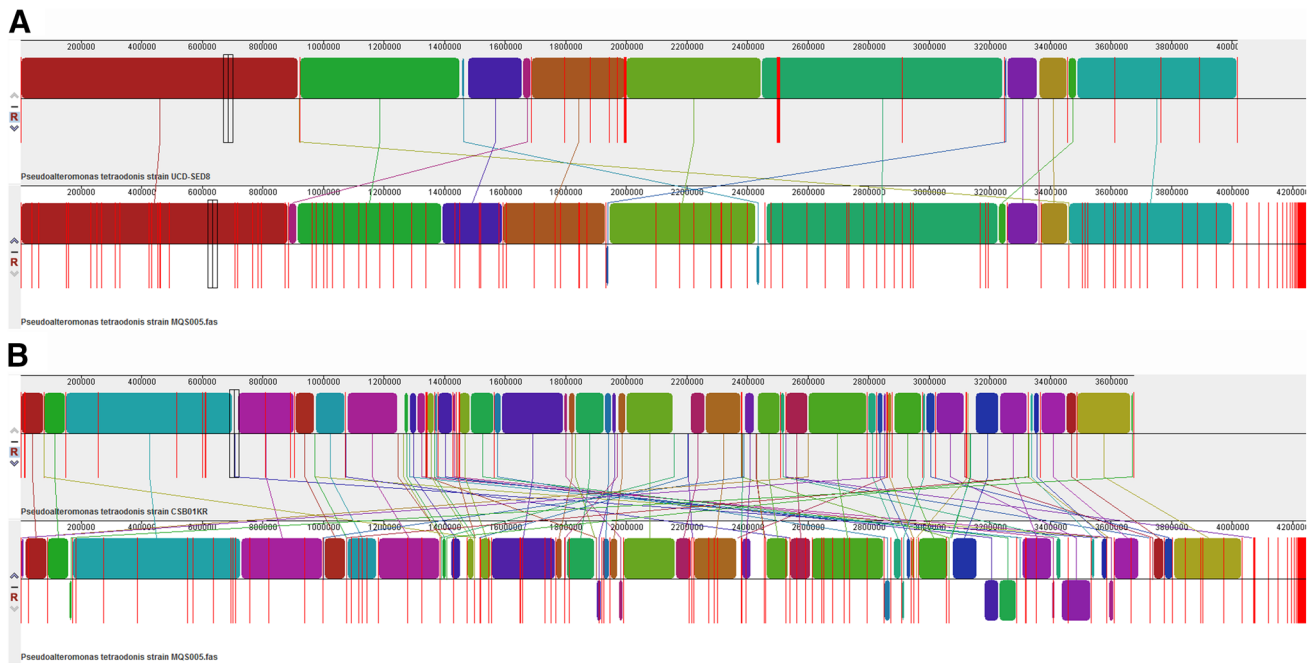


Fig. 1 Reordered draft genome alignments of *P. tetraodonis* strain MQS005 (**a**) to UCD-SED8 (**b**) and CSB01KR. For each figure, the color blocks on top is the reference genome and the color blocks on bottom is our genome of MQS005. The colored blocks are connected

by lines, which shows the regions in each genome are homologous. The vertical red lines between two colored blocks are the regions of sequence contigs/scaffolds (Color figure online)

genes related to AHLs and total of nine potential target genes was found (Table S2). Finally, a gene of Acyl-homoserine-lactone acylase was included and located on scaffold 17.

Structure Analyses of Target Gene

To find the target gene, the structure of target sequence region including the target gene, upstream and downstream elements was analyzed. Between the target gene of APTM01, a gene of beta-lactamase domain protein was downstream in the forward direction and a gene of ribosomal protein S12 methylthiotransferase was upstream in reverse direction (Fig. 2a). When the gene sequence was aligned to published nucleotide sequences on NCBI databased, only two sequences from whole-genome sequences of *Pseudoalteromonas* species (*P. issachenkonii* strain KCTC 12958 and *Pseudoalteromonas* sp. SM9913) were identified by 97%. Then the translated protein sequences were aligned to published nucleotide sequences on NCBI databased, many sequences from other strains of *Pseudoalteromonas* species were identified. This gene belonged to N-terminal nucleophile (Ntn) hydrolase superfamily and contained a conserved domain of penicillin amidase (or penicillin amidases) and acyl-homoserine lactone acylase. But the all

matched sequences in high identity (> 90%) were annotated to putative genes in *P. tetraodonis* and the functions of these genes were not validated. The conserved regions of protein homologous sequences (acyl-homoserine lactone acylase) were shown by alignments (Fig. 2b). The result of multiple sequence alignment showed 4 highly conserve regions including residues 24–44, 83–94, 253–289 and 300–325. Then, the all-protein sequences (containing the target protein sequence) of AHL acylases on UniProtKB/Swiss-Prot (<http://www.uniprot.org/>) were multi-aligned by ClustalX and clustered to analyze their phylogenetic relationship (Fig. 3). On the phylogenetic tree, the target protein was more likely to PvdQ type acylase because it contains the PvdQ domain. But it was more different to other known PvdQ protein. PvdQ gene in *P. aeruginosa* catalyzes the deacylation of acyl-homoserine lactone (longer-chain acyl-HSLs of 11 to 14 carbons in length), releasing homoserine lactone (HSL) and the corresponding fatty acid [13, 43, 44].

Heterologous Expression and Function Verification of Target Genes

The whole-gene sequence containing 2292 bp was amplified by PCR from the whole-genome DNA. With an adaptor, the amplified gene was ligated to the vector pET15b

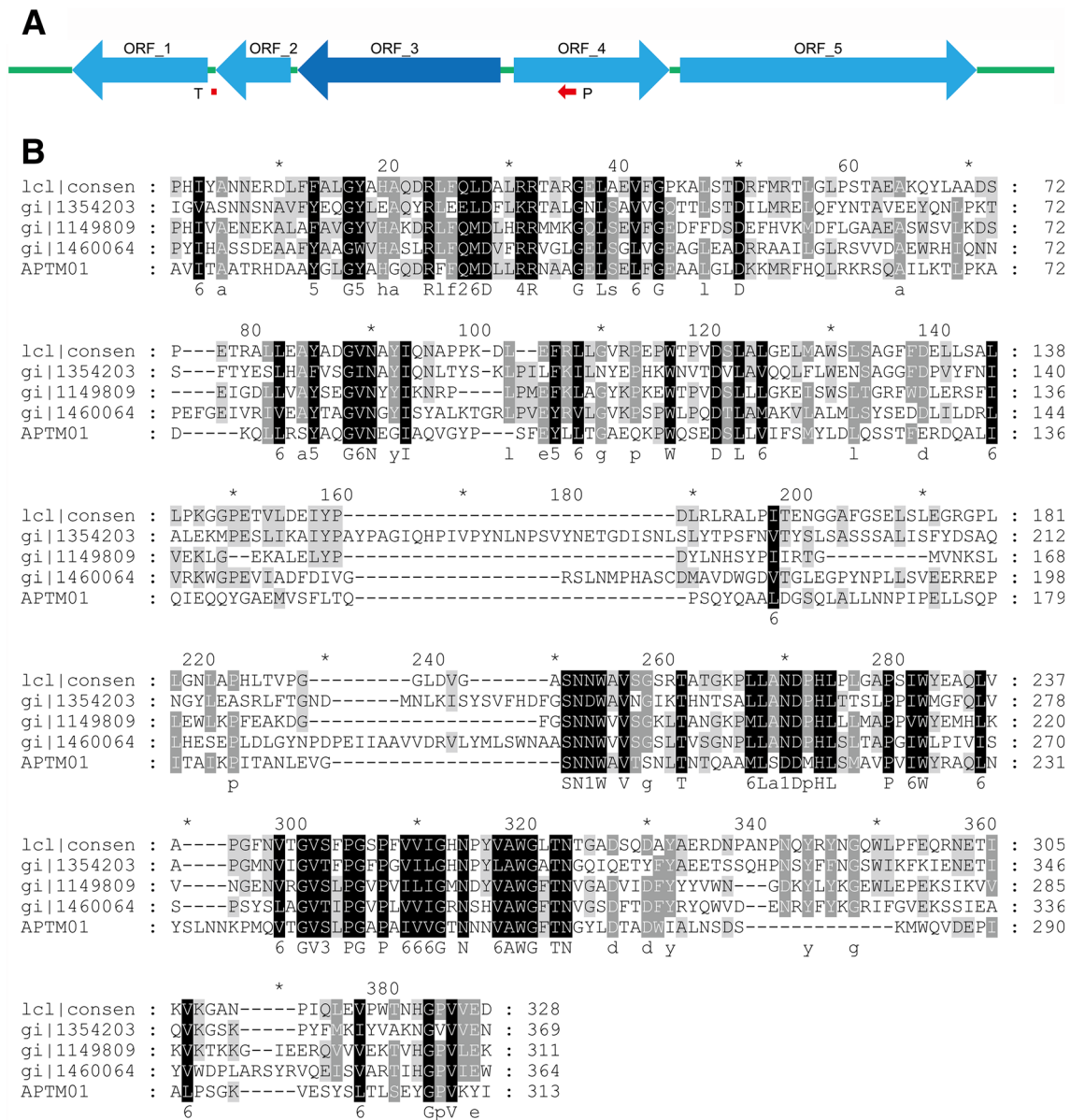


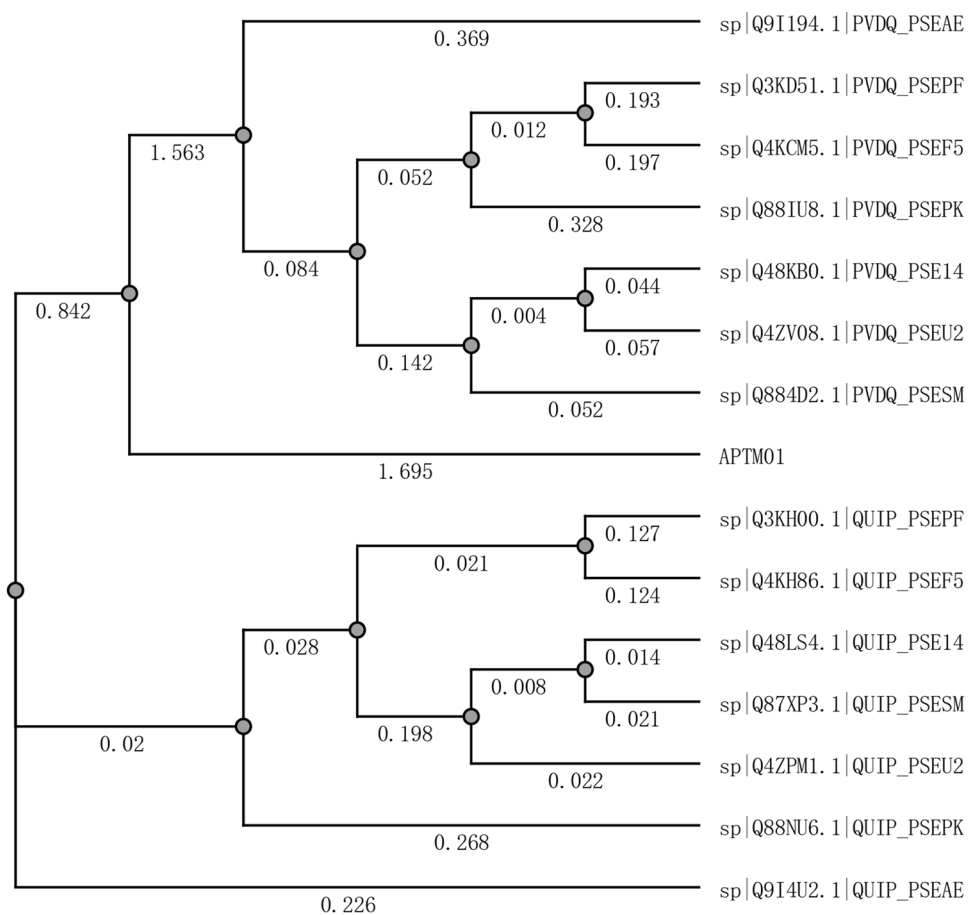
Fig. 2 Location of target gene on genome assembly and alignment of target protein to homologous acyl-homoserine-lactone acylases. **a** Upstream and downstream structure between the candidate gene on scaffold 17. The large arrows are annotated or predicted ORFs; the smaller arrow labeled by “P” is predicted promoter region; and the small block labeled by “T” is predicted terminator region. The ORF_3 is the target gene APTM01; ORF_1 is predicted amidohydrolyase gene containing 1533 bp; ORF_2 is beta-lactamase domain

protein gene containing 849 bp; ORF_4 is ribosomal protein S12 methylthiotransferase containing 1761 bp; ORF_5 is chemotaxis protein CheY gene containing 3354 bp. **b** Multi-alignment of APTM01 protein sequence and similar protein sequences of Acyl-homoserine-lactone acylases. The conserved region is shown by GeneDoc software at 4 levels. Only the region contained conserved sequences was shown

and introduced into the competent cells. The amplified gene was checked with agarose gel electrophoresis and DNA sequencing to obtain the target transformant with correct gene fragment. The vector pET15b-APT01 connected the target AHL acylase gene was introduced into the expressing host of *E. coli* BL21. Because the strain

of *P. tetradonidis* MQS005 could degrade 4 AHLs including C8-HSL, C10-HSL and C12-HSL and OC12-HSL, the expressing host with target gene fragment was validated by degrading these four AHLs. It's obvious that C12-HSL and OC12-HSL were degraded completely by the expressing host with pET15b-APT01 because of the tube did not

Fig. 3 Phylogenetic tree based on amino acid sequences of AHL acylases on UniProtKB/Swiss-Prot. The dendrogram was constructed by the neighbor-joining method using the ClustalW program. The identified AHL acylases were cluster to types including PvdQ and QuiP protein. The target protein belonged to PvdQ type



show purple (Fig. 4). It's debatable that the result color of degrading C10-HSL showed light purple, resulting in the AHLs-diffusion method on punched reporter plate was used to detect the residual C10-HSL. The reporter plate results showed that no diffusion purple area was expended, which indicated that C10-HSL was efficiently degraded by the host with pET15b-APT01 expressing the target gene (Fig. 4A–C). However, the expressing host did not show any degrading activity of C8-HSL because the treatment tube still showed dark purple (Fig. 4).

Discussion and Conclusions

In this study, we screened more than 40 stains of *Pseudoalteromonas*, only 9 strains have different AHL-degrading activity, respectively without AHL production, and the others did not produce AHLs and did not show the ability of AHL degradation. The distribution of AHLs' degrading ability of the *Pseudoalteromonas* genus indicates that this ability is not essential for the survival of these strains. These nine strains have a widely AHL-degrading ability, implicating they are potential resources to explore new

quorum quenching enzymes. Among the strains, some of them, such as MQS004, MQS005, NQ24 and D5204, could degrade more AHLs efficiently and showed more wide AHLs-degrading effect, which implicated that the degrading enzymes in different strains recognized different AHLs and affected different sites of substrate molecule. However, OC8-HSL and OC10-HSL were not be degraded by any screened strains, suggesting that groups of (3-oxo) were an important site affecting the recognition and degrading for the enzymes. For the sequenced two strains, no AHL receptor or regulator gene were annotated. Hence, AHLs may be only as common nutrient substance rather than signal molecules in these two strains. It is interesting that some strains, such as HK5, NQ24 and D2139 secreted other longer-chain AHLs after induced by short-chain AHLs. When these strains receipted the single of short-chain AHLs from the environment, it's possible that they also expressed other relative genes. And then the secreted longer-chain AHLs would be a new signal to affect the environment. This process of single reception and sending might be a new mechanism of accurate interactions among environmental bacteria whether the same or different individuals.

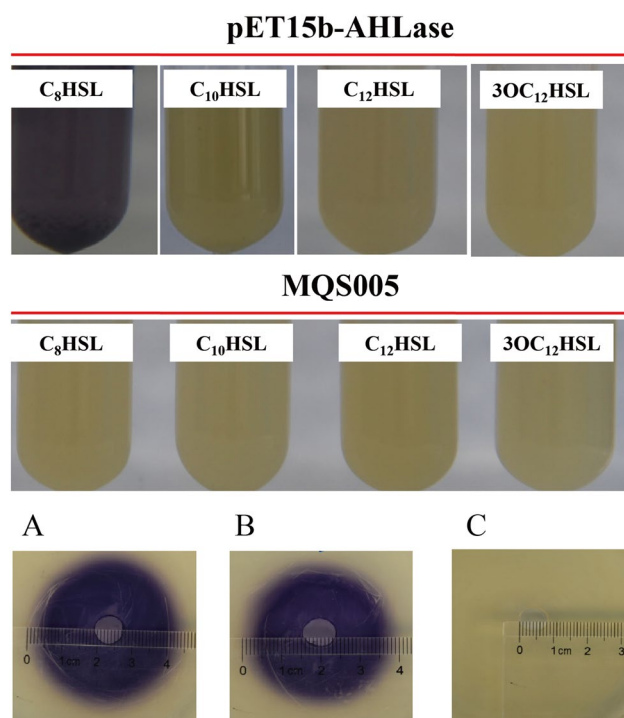


Fig. 4 AHL-degrading ability of pET15b-APT01. A, B and C for C10-HSL. A Control without degradation of any hosts; B degradation of the host with the vector of pET15b; C degradation of the host with the vector of pET15b-APT01

We found a potential acylhomoserine-lactone acylase gene from *P. tetraodonis* strain MQS005 with the method of whole-genome sequencing. The upstream and downstream ORFs between the target gene APTM01 were analyzed. Promoters and terminators were also predicted by BPROM and FindTerm (<http://www.softberry.com/>) [45], respectively. The predicted result showed that the nearest promoter is in the nearest upstream and encode region and the nearest terminator was in the noncoding region following the nearest downstream gene (Fig. 2), which indicate that the transcription unit contained multiple genes. Four conserved regions which identified by the multiple alignments of published acylhomoserine-lactone acylases and APTM01 exist, which indicate that these regions contain potential functional structure(s) for this type acylases.

The cloned target gene APTM01 belongs to Ntn hydrolase superfamily and contains a conserved domain of AHL acylase which has a definite domain (<https://www.ncbi.nlm.nih.gov/cdd/>, ID is COG2366). Therefore, the acylhomoserine-lactone acylase gene was identified and annotated according to the sequences in public databases. The

cloned gene was assigned to PvdQ type but much different from other known PvdQ type genes in the phylogenetic tree based on amino acid sequences. The information of this gene would be added to the public database of GenBank and help to explore new similar AHL acylase. In contrast, the acylhomoserine lactonase gene in widely species did not contain a definite conserved domain in the database and were very different from each other in different species, which result in no sequence pattern to screen new acylhomoserine lactonase genes (Fig. 5). In most previous researches, genomic libraries were constructed to explore the acylhomoserine lactonase genes as quorum quenching enzymes [21, 46–49] though the same genus contained the similar protein sequence [50].

The constructed vector with the AHL acylase gene APTM01 could efficiently degrade C10-HSL, C12-HSL and OC12-HSL except C8-HSL, but the original strain of MQS005 could degrade these 4 AHLs. The difference could result from the incorrect fold of the target protein in heterogenous host or the existence of other degrading gene(s) in the genome of *P. tetraodonis* strain MQS005.

AHLs as a signal molecule in bacterial quorum sensing system transform the information among the individuals in the environment, which has been shared recognition. However, more other substances, such as secondary metabolites and environmental stress factor, could induce the expression of specific genes to show similar properties to signal molecules. In closed environments, are signal molecules degraded after they are transferred into the bacterial cell and induce the expression of specific genes? If yes, the concentration of signal molecules will decrease until to stable state as the degrading, which shows a kind of feedback adjustment. If not, the concentration of signal molecules will be a “switch” to regulate relative genes’ expression.

In conclusion, bacteria of *Pseudoalteromonas* had wide-activity of degrading AHLs. We had obtained nine strains which have different AHL-degrading activity through screening marine bacteria in large scale. These strains will be resources of exploring quorum quenching enzymes. And an AHL acylase gene of new PvdQ type in the sub-branch of phylogenetic tree has been found from the strain MQS005 by whole-genome sequencing and its functions were verified. Comparing to the method of screening genomic fragment library, whole-genome sequences screening is more efficient and inexpensive though similar genes is required. With the development of transcriptome sequencing technology, bacterial transcriptome sequencing will be also employed to explore and obtain new genes of degrading single molecules efficiently. More relative domain of

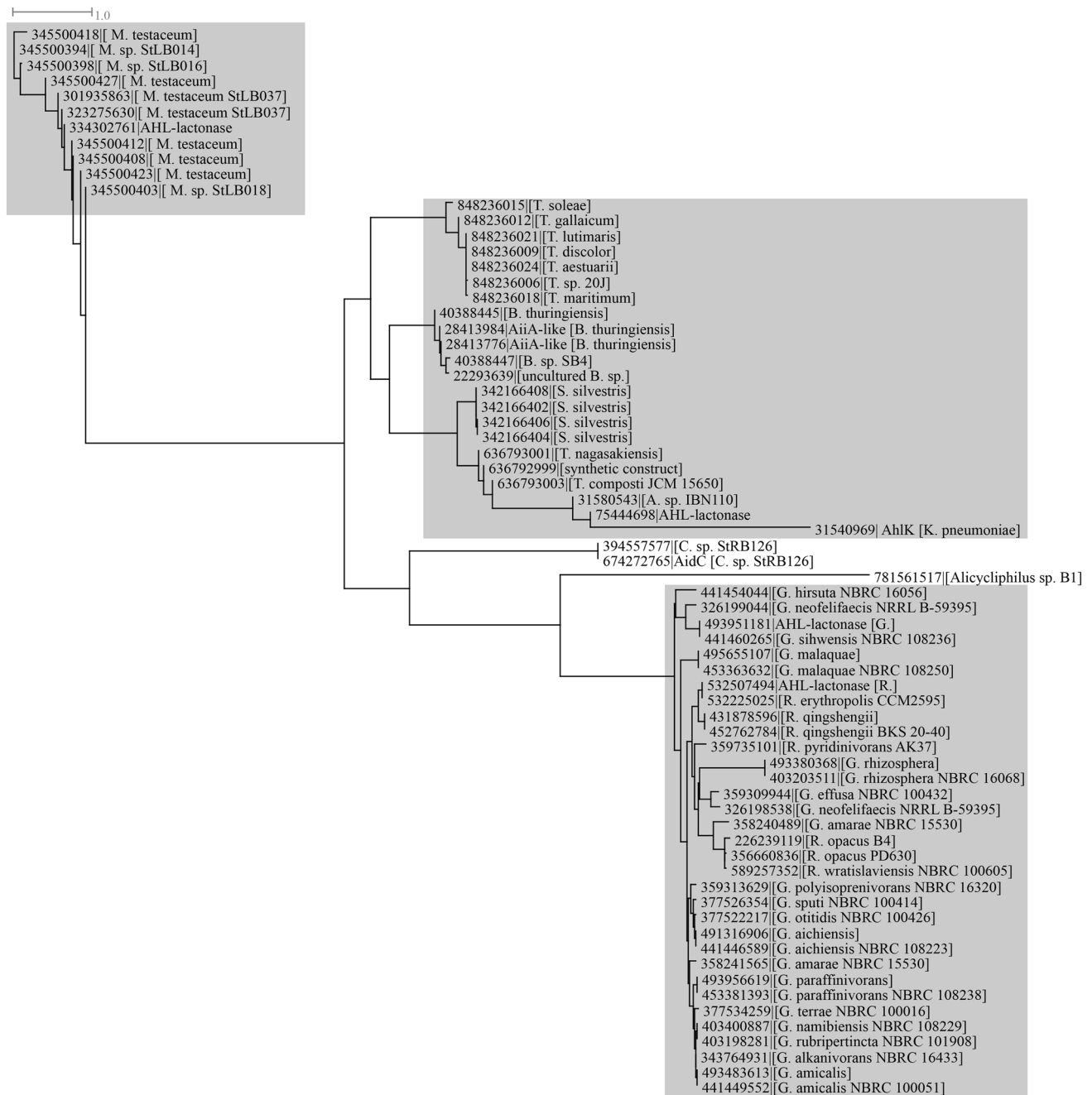


Fig. 5 Dendrogram for all definite acylhomoserine lactonase in various species. All the protein sequences were from public databases and not putative protein

AHLs-degrading activity will be annotated with the public database updating, which would help to explore more quorum quenching genes rapidly. The obtained AHL acylase gene would be a potential quorum quenching gene to apply in many fields.

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Compliance with ethical standards

Conflict of interest We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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