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Full Length Research Paper

Identification of *Vibrio cholerae* as a causative bacterium for an ulcer disease of cultured loach *Misgurnus anguillicaudatus* in China

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A bacterial disease was reported from cultured loach *Misgurnus anguillicaudatus* in China. Symptoms included haemorrhages at the body surface (including base of fin, head, mouth, and abdomen), ulceration of muscle, and high-level mortalities. The dominant bacterial colonies were isolated in nutrient agar from different infected body parts and confirmed the pathogenicity by challenge experiments. The 3 strains (NQ1-3) were randomly selected and characterized phenotypically and genotypically. Biochemically, the 3 strains showed properties and biochemical characteristics similar to *Vibrio cholerae*. Phylogenetic analysis of the 16S rRNA gene (GenBank accession No. JF939043) and 3 housekeeping genes, RNA polymerase a-chain (*rpoA*, GenBank accession No. JF939042), recombination repair protein (*recA*, GenBank accession No. JF939045) and DNA gyrase B subunit (*gyrB*, GenBank accession No. JF909344) of strain (NQ1) exhibited high similarity to those of *V. cholerae* from GenBank. The 3 strains (NQ1-3) were positive for *toxR*, *lolB* (previously called *hem*M) and *rtxA* (presumptive cytotoxin) genes, potential virulence factors. To sum up, the isolated strains were confirmed as *V. cholerae* on the basis of phenotypic characteristics, phylogenetic analysis of the 16S rRNA gene and three housekeeping genes, as well as potential virulence factors studies. The susceptibility of isolates to 28 antimicrobial agents was determined, NQ1 were found to be sensitive to some of the drugs tested, including carbenicillin, azlocillin, cefoperazone, ceftazidime, ceftriaxone, cephradine, azithromycin, lomefloxacin, levofloxacin, enoxacin, norfloxacin, neomycin, streptomycin, doxycycline, tetraecycline, and minomycin.

Key words: *Misgurnus anguillicaudatus*, *Vibrio cholerae*, housekeeping genes, ulcer disease.

INTRODUCTION

Misgurnus anguillicaudatus of the order Cypriniformes is a small freshwater teleost, it has been reared under farming conditions in China since 1950s as a delicious fish with high nutritional value and as traditional Chinese medicine. *M. anguillicaudatus* was widely cultured in the Jiangsu province of China, making a great profit on economics in recent years. Owing to the intensive culture, bacterial infectious diseases often occurred between May and October each year and resulted in serious losses (Zhang et al., 2010; Yao et al., 2010). In August 2010, an infectious disease occurred as outbreaks of high mortality, which was responsible for important economic losses in intensive culture of loach in some farms of Dunshang county of Jiangsu province (China). Moribund loach showed typical signs of external haemorrhages and ulceration of muscle. Moribund loach collected in the pond were found to be infected by a dominant bacteria, the pathogenicity of this bacteria has been determined by challenge experiments, and phenotypic characteristics, phylogenetic analysis of the *16S rRNA* gene and three housekeeping genes (*rpoA*, *recA*, *gyrB*), as well as examination of specific genes

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showed that this dominant bacterial isolate was similar to *V. cholerae*.

V. cholerae is a gram-negative bacterium which comprises part of the autochthonous microflora of aquatic environments, sea water and fresh water bodies are the main reservoirs for *V. cholerae* (Lipp et al., 2003); it has been detected on the surfaces of aquatic organisms such as zooplankton, phytoplankton, insects, crustaceans, and plants (Colwell and Huq, 1994). Of medical importance, however, is that certain members of the species have caused the severe life-threatening diarrheal disease cholera (Hoge et al., 1996; Kam et al., 1995; Yuen et al., 1994; Dalsgaard et al., 1999), and some non-O1/non-O139 *V. cholerae* strains were known to be responsible for infections of aquatic animals (Haldar et al., 2007; Muroga et al., 1979, Kiiyukia et al., 1992; Reddacliff et al., 1993; Yang et al., 2006; Zheng, 1986). Several virulence genes are reported to be present in *V. cholerae.* Although CT is supposed to be the major factor involved in the pathogenicity of *V. cholerae*, a number of other genes are also critical for establishing a productive infection, these include toxin regulatory protein gene (*toxR*) and *rtxA* (presumptive cytotoxin) gene (Miller and Mekalanos, 1988; Lin et al., 1999). The objective of this study is to explore *V. cholerae* as the pathogen of high-level mortality of loach and the etiological role of this bacteria in aquaculture.

MATERIALS AND METHODS

Diseased loach and symptoms

Diseased loach, collected from loach farms, located in Dunshang County, Jiangsu province, P.R.China in July 2010. Average body length of the infected fish was 12 cm. Approximately 30% loach were affected initially and in later stage it spread up to 70%. Symptoms of the disease include gradual erosion of muscle with red coloration, haemorrhages at the body surface (base of fin, head, mouth and abdomen). The infected fishes were always swimming on the surface and frequent blood clots were also found in body surface. High level of mortality was recorded within one week.

Isolation of bacteria

The surface of diseased loach was sterilized with 70% ethanol. Samples were collected aseptically from the liver, and muscle tissues and streaked onto nutrient agar and blood agar plates, and incubated at 28°C for 24 h. After incubation, three morphologically similar and dominant bacterial colonies (No.NQ1-3) were randomly selected and inoculated onto nutrient agar slants, cultured for 24 h at 28°C, stored at 4°C and subjected to Gram staining, motility, physiological and biochemical tests for further identification. Meanwhile, all the isolates were stored in nutrient broth supplemented with 10% glycerol at -70°C.

Experimental infection of healthy loach

A representative strain (NQ1) was used in challenge experiment to confirm pathogenicity. Before onset of experiments, loach from a healthy farms located in Lianyungang city of Jiangsu province, China, with average body length of 10 cm were purchased and acclimatized with sterile water for one week before commencement of the experiment at room temperature. The challenged fishes were injected intraperitoneally with 0.1 ml volumes of live cells (10^4 to 108 $CFUml^{-1}$) per fish. Inoculated fishes were cultured at 20°C and observed daily during 10 days. All mortalities were recorded, and dead and moribund fishes were removed for pathological examination, external and internal signs of disease were recorded, necropsied samples of the liver and muscle were streaked onto nutrient agar plates and blood agar plates for bacteriological examination. In challenge experiment, a control group consisted of an identical experimental setup but without the pathogenic bacterium.

Pathogenicity was confirmed by occurrence of disease, clinical signs and morbidity congruent with those of natural causes, and same strains were reisolated from the moribund and dead loach to prove the Koch postulates.

Phenotypic tests

The tested strains (NQ1-3) were subjected to the following phenotypic tests: Gram stain, oxidase activity, cell morphology and motility, oxidation/fermentation test, mannitol and sucrose, gas and acid production from glucose, indole, methyl red, Voges-Proskauer reaction, utilization of citrate, dihydrolation of arginine, decarboxylation of lysine, nitrate reduction, and use of compounds as unique carbon sources, salt tolerance tests (0, 1, 3 and 6% NaCl), and growth on thiosulfate citrate bile salts sucrose (TCBS) agar. Sensitivity to the *Vibrio* static agent O/129 (2, 4-diamino-6, 7-diisopropylpteridine, 150 and 10 µg per disc, respectively) was determined.

Sequencing of 16S rRNA and housekeeping genes, and phylogenetic analyses

A representative strain (NQ1) was selected for molecular identification by *16S rRNA*, *rpoA*, *recA* and *gyrB* genes. Polymerase chain reaction (PCR) amplification was performed in a total volume of 20 µl containing the appropriate reaction buffer and reagents: 0.2 µl (2.5 U/µl) *Taq* DNA polymerase (Sigma), 0.2 µl (10 µM) forward and reverse primers respectively, 0.4 µl dNTP (200 mM), 1.6 µl (1.5 mM) $MgCl_2$, 2 µl 1×PCR buffer, 14.4 µl distilled water, 40 ng template DNA.

Universal PCR primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGM TAC CTT GTT ACG ACT T-3') were used for amplification of the *16S rRNA* gene (Martin and Collen, 1998). The thermal cycling protocol used included initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 45 s and extension at 72°C for 1 min. A final extension step of 72°C for 7 min was also used.

Universal PCR primers UP1 (5'-GAA GTC ATC ATG ACC GTT CTG CAY GCN GGN GGN AAR TTY GA-3') and UP2r (5'- AGC AGG GTA CGG ATG TGC GAG CCR TCN ACR TCN GCR TCN GTCAT-3') were used for amplification of the *gyrB* gene (Yamamoto and Harayama, 1995). The thermal cycling protocol used included initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 2 min. A final extension step of 72°C for 7 min was also used.

Universal PCR primers *rpoA* F (5'-ATG CAG GGT TCT GTD ACA G-3') and *rpoA* R (5'-GHG GCC ART TTT CHA RRC GC-3') were used for amplification of the *rpoA* gene (Thompson et al., 2005). The thermal cycling protocol used included initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1

min, annealing at 52°C for 1 min and extension at 72°C for 1 min. A final extension step of 72°C for 7 min was also used.

Universal PCR primers *recA* F (5'-TGG ACG AGA ATA AAC AGA AGG C-3') and *recA* R (5'- CCG TTA TAG CTG TAC CAA GCG CCC-3') were used for amplification of the *recA* gene (Thompson et al., 2004). The thermal cycling protocol used included initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55.5°C for 30 s and extension at 72°C for 1 min. A final extension step of 72°C for 7 min was also used.

PCR products were purified using a DNA purification system (Wizard PCR Preps, Promega) and sequenced by Biotechnology Corporation, Shanghai, China. The *16S rRNA*, *rpoA*, *recA* and *gyrB* sequences determined were compared against other sequences using the BLASTN search algorithm (National Center for Biotechnology Information, NCBI) to determine the closest matching sequences in GenBank and to infer possible phylogenetic affiliations. These *16S rRNA*, *rpoA*, *recA* and *gyrB* sequences (both the determined and reference sequences) were aligned using CLUSTAL X version 1.83 program (Thompson et al. 1997). Interspecies/interstrain similarity for each gene was determined using MEGA III software (Kumar et al., 2004). Phylogenetic trees were constructed using the neighbour-joining method. Distance matrices were calculated using Kimura's 2-parameter distances. Robustness of topologies was assessed by the bootstrap method with 1000 replicates. Final classification of *16S rRNA*, *rpoA*, *recA* and *gyrB* genes to a phylogenetic division or subdivision, was based on combined results from the phylogenetic group represented by the closest matching sequences in the GenBank and phylogenetic tree analyses. In addition, the NQ1 *16S rRNA* sequence was compared to sequences in the Ribosomal Database Project using BLAST (Altschul et al., 1990) to confirm the genus assignment of NQ1.

Detection of *toxR*, *lolB* and *rtxA* genes

Three genes were detected by a specific PCR assay using the following primers: *toxR*-F (5'-GTC TTC TGA CGC AAT CGT TG-3'), *toxR*-R (5'-ATA CGA GTG GTT GCT GTC ATG-3') for *toxR* as previously described (Rivera et al., 2001); *rtxA*-F (5'-CTG AAT ATG AGT GGG TGA CTT ACG-3'), *rtxA*-R (5'-GTG TAT TGT TCG ATA TCC GCT ACG-3') for *rtxA* as previously described (Chow et al., 2001); *lolB*-F (5'-TGG GAG CAG CGT CCA TTG TG-3'), *lolB*-R (5'-CAA TCA CAC CAA GTC ACT C-3') for *lolB* as previously described (Lalitha et al., 2008).

Detection of antimicrobial susceptibility of isolates

Antimicrobial susceptibility was performed by disk diffusion test according to the manufacturer's instruction from Hangzhou Tianhe Microorganism Reagent Co., Ltd. and standard methods for aquatic animal bacteria pathogen susceptibility testing recommended by Alderman and Smith (2001). The Mueller-Hinton agar and broth, antimicrobial agents were purchased from Hangzhou Tianhe Microorganism Reagent Co., Ltd., China.

The inoculum (NQ1) after overnight culture was standardised to have a concentration of 2-3×10^8 CFU/ml (McFarland opacity 0.5 standard). Bacterial suspension (0.1 ml) was spread onto Mueller-Hinton agar with sterile cotton swabs and antimicrobial agent discs were then added. Plates were then incubated at 28°C for 48 h. The diameters of each zones of inhibition were read two times at 24 and 44 h, respectively. Agar plates inoculated only with the tested strain NQ1 without introduction of antimicrobial discs served as controls. Analysis for each combination of the tested strain NQ1 and antimicrobial discs were repeated three times. For each reading, the diameter of the zone of inhibition was assessed by two measurements at right angles to one another. Characterization of strains as resistant, intermediate, or sensitive was based on the size of the inhibition zones around each disc according to standards suggested by of explanation provided by Hangzhou Tianhe Microorganism Reagent Co.

RESULTS

Isolation of bacteria

Bacteriological cultures on nutrient agar and blood agar from the liver and muscle of moribund loach yielded an apparently pure culture. Three strains isolated from liver and muscles were randomly selected, which were numbered: NQ1-3.

Pathogenicity of isolates

Experimental infection reproduced the apparently identical disease syndrome observed in the farms. All of injected fish with representative strain (NQ1) died at 2 to 5 day post-injection, especially in the 10^6 to 10^8 CFU/ml concentration; the strain (NQ1) caused 100% mortality of experimental loach within two days. In the experiment, it was found that an overwhelmingly dominant strain was isolated from the livers and body ulcers of the moribund fish. Thus results demonstrated that the isolates were the causal agent of the episode of mortalities of loach. No deaths or visible changes in the control groups were observed, and no bacteria were isolated from these fish.

Phenotypic characteristics

The morphological characteristics of 3 isolated strains (NQ1-NQ3) were identical, and shared the properties of *V. cholerae* (Table 1). They were Gram-negative, fermentative slightly curved rods with round-ends, approximately 1.5–2.5 µm long and 0.8–1.0 µm wide, which were motile by single polar flagella. Oxidase, catalase, lysine decarboxylase, β-galactosidase were produced, but not arginine dihydrolase and H_2S. Nitrates were reduced. The methyl red test and Voges Proskauer reaction were positive. Sensitivity was demonstrated to the vibriostatic agent, O/129 (150 and 10 µg per disc, respectively). The three strains tested grew at 28°C producing a translucent greyish-white lawn of moderate growth on nutrient agar plates, and with a yellow lawn (ferment sucrose) on thiosulphate citrate bile salt sucrose agar (TCBS).

Multilocus sequence analysis

Several genes (*16S rRNA*, *rpoA*, *recA* and *gyrB*) have been used to infer phylogenetic relationships. The length and accession number of the *16S rRNA*, *rpoA*, *recA* and

Table 1. The characteristics of NQ1-3 in comparison with the *V. cholera*.

Characteristics	NQ1-3	*V. cholerae**	Characteristics	NQ1-3	*V. cholerae**
Growth at 37°C	+	+	ONPG	+	+
Oxidase	+	+	Malonate utilization	+	—
Catalase	+	+	Citrate utilization	+	d
O-F test	F	F	Acetate utilization	+	·
Motility	+	+	Tartrate utilization	—	+
Glucose, acid production	+	+	Mucate utilization	—	—
gas production	—	—	Phenylalanine deaminase	—	—
Lactose	—	—	Trehalose	+	+
Maltose	+	+	Raffinose	—	—
Mannitol	+	+	Fructose	+	·
Mannose	+	d	Melibiose	—	—
Sucrose	+	+	Cellobiose	+	—
Arabinose	—	—	MR test	+	+
Arabitol	—	—	V-P test	+	+
Xylose	—	—	Glucosamine	—	·
Galactose	+	+	H_2S production	—	—
Sorbitol	—	—	Nitrate reduction	+	+
Sorbose	—	·	α-methyl-D-glucoside	—	—
Dulcitol	—	—	Indole	+	+
Erythritol	—	—	Arginine dihydrolase	—	—
Amygdatin	—	·	Lysine decarboxylase	+	·
L-Rhamnose	—	—	Growth at NaCl: 0%	+	+
Oextrin	+	·	1%	+	+
Inositol	—	—	3%	+	·
Adonitol	—	—	6%	—	d
Salicin	—	—	O/129: 10 μg	S	S
Esculin	—	—	150 μg	S	S

Notes: +, positive; —, negative; d, 11%-89% positive; •, not described in references; F, fermentative; S, sensitive. *The data of *V.cholerae* come from Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), Bergey's Manual of Systematic Bacteriology (Brenner et al., 2008, second edition).

gyrB genes were listed in Table 2. The *16S rRNA* sequences of NQ1 displayed 99% similarity to the *16S rRNA* gene of the *Vibrio* strains from the GenBank database. Partial sequences of *rpoA*, *recA* and *gyrB* were determined for the strain (NQ1), and *rpoA* gene displayed 98~99% similarity with published *V. cholerae* strain (EF643483, FJ970909, AJ842581, etc.) from GenBank database; *recA* gene displayed 98~99% similarity with published *V. cholerae* strain (FJ645930, AJ842388, AF117882, etc.); *gyrB* gene displayed 98~99% similarity with published *V. cholerae* strain (DQ386878, HM009682, HM009579, etc.). Phylogenetic analysis based on these sequences, using the neighbour joining, confirmed their position in the genus *Vibrio*, and allocated NQ1 to the *V. cholerae*, the phylogenetic trees based on these sequences, NQ1 clustered with the *V. cholerae* strains(Figures 1 to 4), and was supported by a higher bootstrap value, strongly supporting the assignment of isolate NQ1 to *V. cholerae*. Comparison of the NQ1 *16S rRNA* sequence to sequences in the Ribosomal Database Project database using BLAST also supported the conclusion that NQ1 belonged to *V. cholerae*.

Identification of pathogenic genes

PCR screening revealed that all strains (NQ1-3) exhibited positive PCR results for *lolB* (519 bp), *rtxA* (417 bp) and *toxR* (779 bp) genes, and single specific bands were obtained for all strains after electrophoresis (Figure 5).

Antimicrobial susceptibility

The susceptibility of isolates to 28 antimicrobial agents was determined. The results are given in Table 3. NQ1-3 were found to be sensitive to some of drugs tested,

Table 2. The length and accession number of the *16S rRNA*, *rpoA*, *recA* and *gyrB* genes.

***16S rRNA* sequence**		***rpoA* sequence**		***recA* sequence**		***gyrB* sequence**	
length	accession no.	length	accession no.	length	accession no.	length	accession no.
1449bp	JF939043	916bp	JF939042	849bp	JF939045	1083bp	JF909344

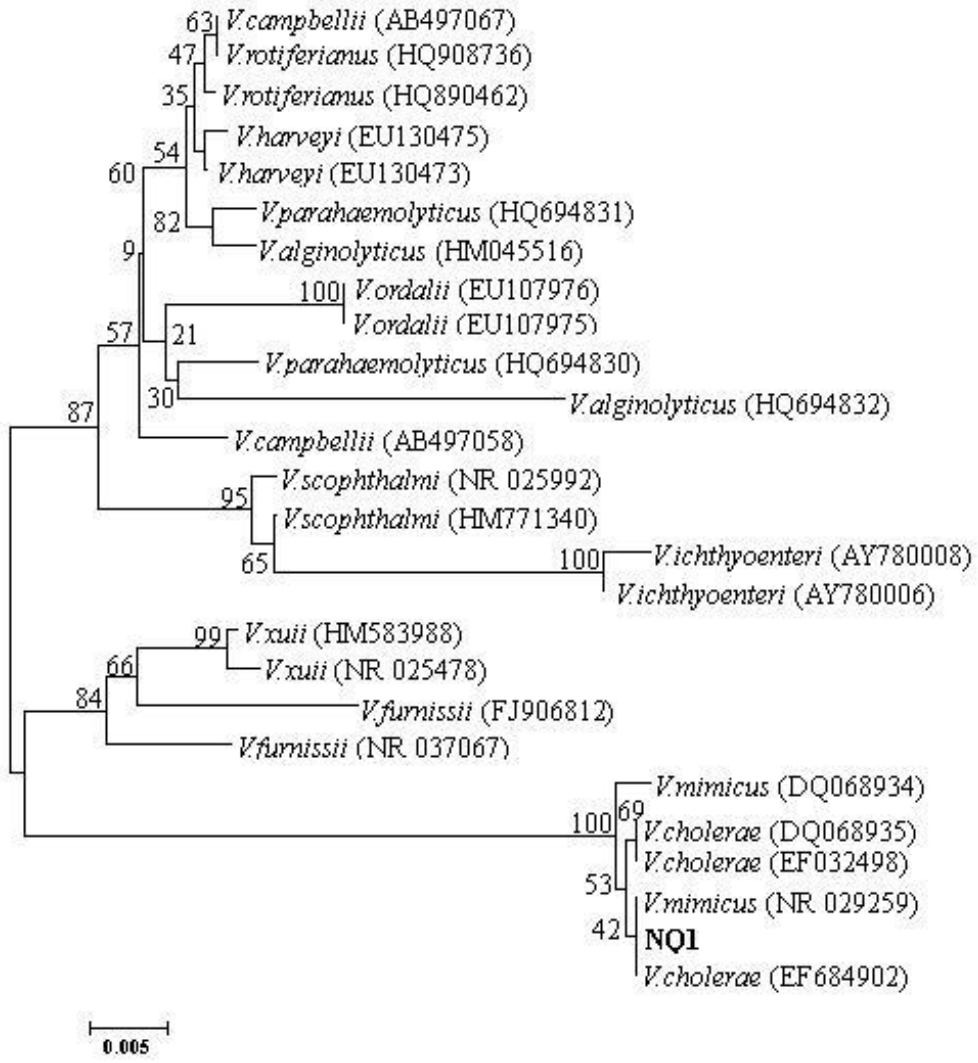

Figure 1. The neighbor joining (NJ) phylogenetic tree based on the partial *16S rRNA* gene sequences (AB497067~ EF684902are database accession numbers in NCBI. Numbers in tree are bootstrap values).

including carbenicillin, azlocillin, cefoperazone, ceftazidime, ceftriaxone, cephradine, azithromycin, lomefloxacin, levofloxacin, enoxacin, norfloxacin, neomycin, streptomycin, doxycyline, tetracycline, minomycin.

DISCUSSION

V. cholerae (O1 and O139) are important human pathogens causing both gastroenteritis and cholera, non-O1/non-O139 *V. cholerae* strains were known to be responsible only for sporadic cases of gastroenteritis and for extraintestinal infections (Morris Jr, 1990), and well recognized as pathogenic bacteria of aquatic animals (Haldar et al., 2007; Muroga et al., 1979; Kiiyukia et al., 1992; Reddacliff et al., 1993; Yang et al., 2006; Zheng, 1986). In our study, dominant bacterial colonies were isolated from diseased loach, and the pathogenicity of

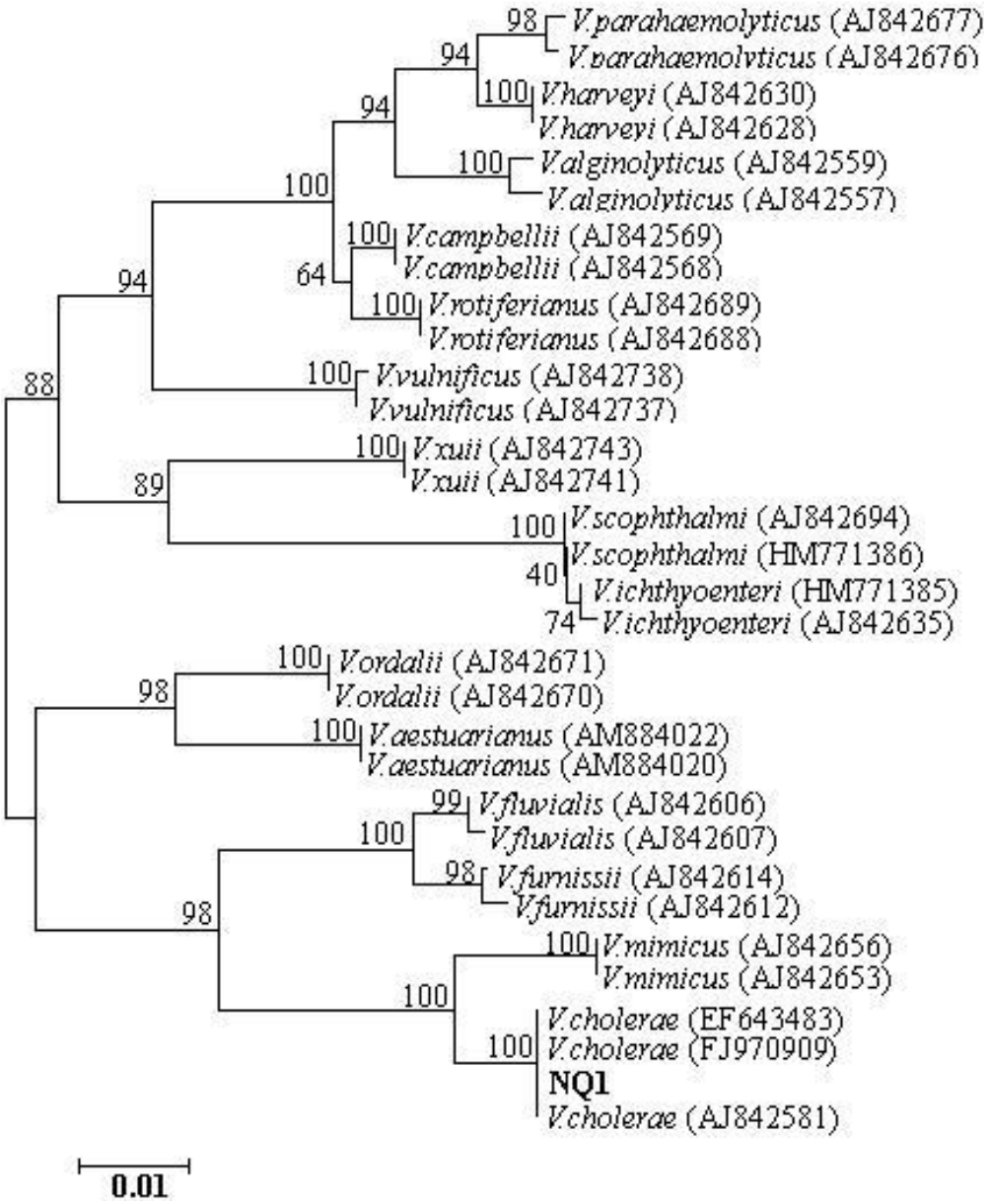

Figure 2. The neighbor joining (NJ) phylogenetic tree based on the partial *rpoA* gene sequences (AJ842567~EU652312 are database accession numbers in NCBI. Numbers in tree are bootstrap values).

representative strain (NQ1) to healthy loach were confirmed using challenge test. The experimentally infected loach all manifested 100% mortality within 2 days in the 10^8 CFU/ml and the 10^7 CFU/ml concentrations, and strong pathogenicity of the isolates to loach was confirmed. The results showed that the isolated dominant bacteria were pathogen of significant mortalities of cultured loach occurred in some farms of Dunshang County of Jiangsu province, China, in August 2010, and were identified as *V. cholerae* based on their morphological, physiological, biochemical and molecular characteristics.

Comparison of the morphological and biochemical characteristics of isolates (NQ1-NQ3) with *V. cholerae*, showed that they shared a high proportion of identical characteristics in classical tests. In addition, we further conducted molecular identification, the *16S rRNA*, *rpoA*, *recA* and *gyrB* genes were used as the molecular markers for bacterial species identification in this study. The *16S rRNA* sequence of isolate (NQ1) displayed very high levels of sequence similarity (99%) to the *16S rRNA* gene of *Vibrio* spp. From GenBank, but it did not

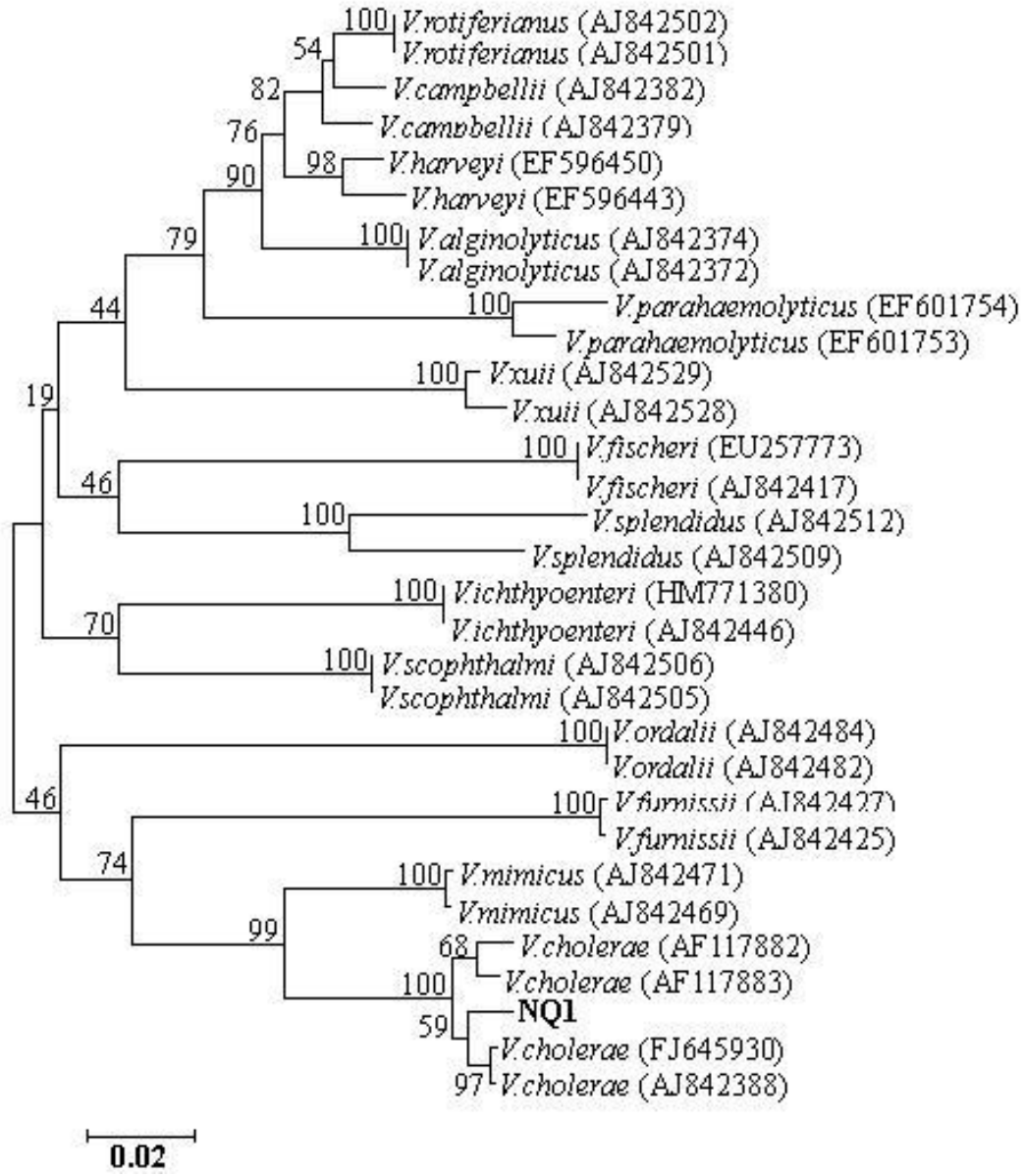

Figure 3. The neighbor joining (NJ) phylogenetic tree based on the partial *recA* gene sequences (AJ842502~AJ842388 are database accession numbers in NCBI. Numbers in tree are bootstrap values).

essential for bacterial replication. The *rpoA*, *recA* and *gyrB* gene sequences of NQ1 displayed high similarity (98~99%) with that of published *V. cholerae* strains from GenBank database, respectively. NQ1 clustered with the *V. cholerae* strains, and was supported by a bootstrap value of 100% in Figures 2 to 4, strongly supported that the NQ1 belong to *V. cholerae*.

Although CT is supposed to be the major factor involved in the pathogenicity of *V. cholerae*, non-O1/non-O139 type of *V. cholerae* which do not possess either the ctx operon or other virulence genes, but can still cause cholera-like diarrhoea and were responsible for sporadic and localized outbreaks (Kaper et al., 1995; Vital et al., 2002). The *toxR* gene was first discovered as the regulatory gene of the cholera toxin operon, but it was later shown to be involved in the regulation of many other genes in *V. cholerae* (Miller et al., 1987), and the *toxR* gene appears to be well conserved among *Vibrio* species; the *rtxA* gene encodes the presumptive cytotoxin, which are proven to be associated with cytotoxicity in HEp-2 cells (Lin et al., 1999; Chow et al., 2001); the *lolB* gene that could best serve as a reliable molecular marker for the detection of all biotypes and O1 and O139 and non-O1/non-O139 serogroups of *V.*

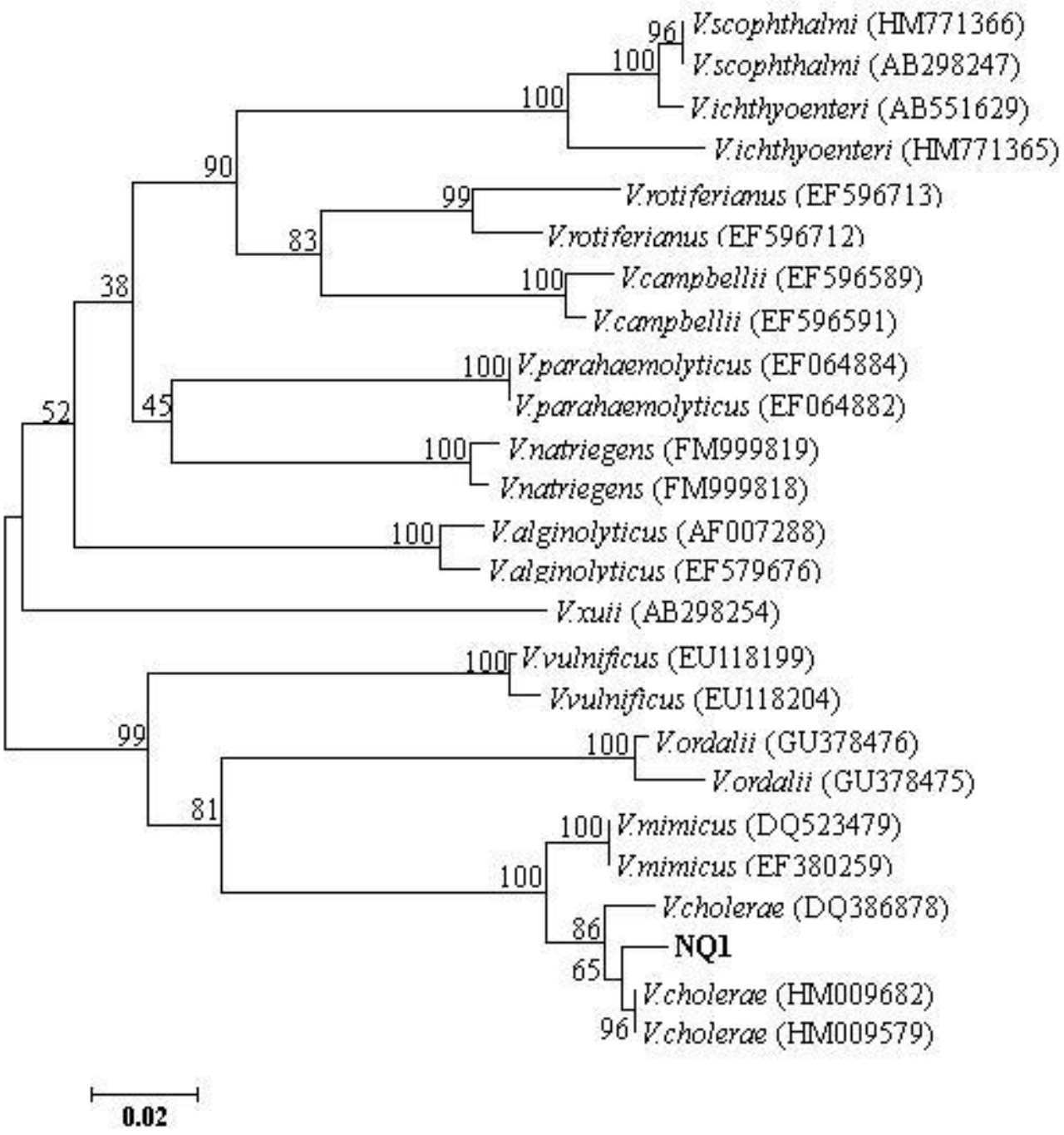

Figure 4. The neighbor joining (NJ) phylogenetic tree based on the partial *gyrB* gene sequences (AJ842677~AJ842581 are database accession numbers in NCBI. Numbers in tree are bootstrap values).

cholerae. Therefore, we analyzed the tested strain (NQ1) by PCR for the presence of these genes (*toxR* gene for 779bp, and *rtxA* gene for 417bp, the *lolB* gene for 519bp) that are known in *V. cholerae*. The PCR assay has demonstrated that all strains (NQ1-3) exhibited positive PCR results for *lolB*, *rtxA* and *toxR* genes, further supporting the assignment of isolate (NQ1-3) to *V. cholerae* and its pathogenicity.

The resistance of isolates to antimicrobial agents is remarkable (Table 3). That of 28 antimicrobial agents, the isolates were resistant to penicillin G, ampicillin, oxacillin, amoxicillin, clarithromycin, clindamycin, erythromycin, trimethoprim/sulfamethoxazole, and susceptible to carbenicillin, azlocillin, cefoperazone, ceftazidime,

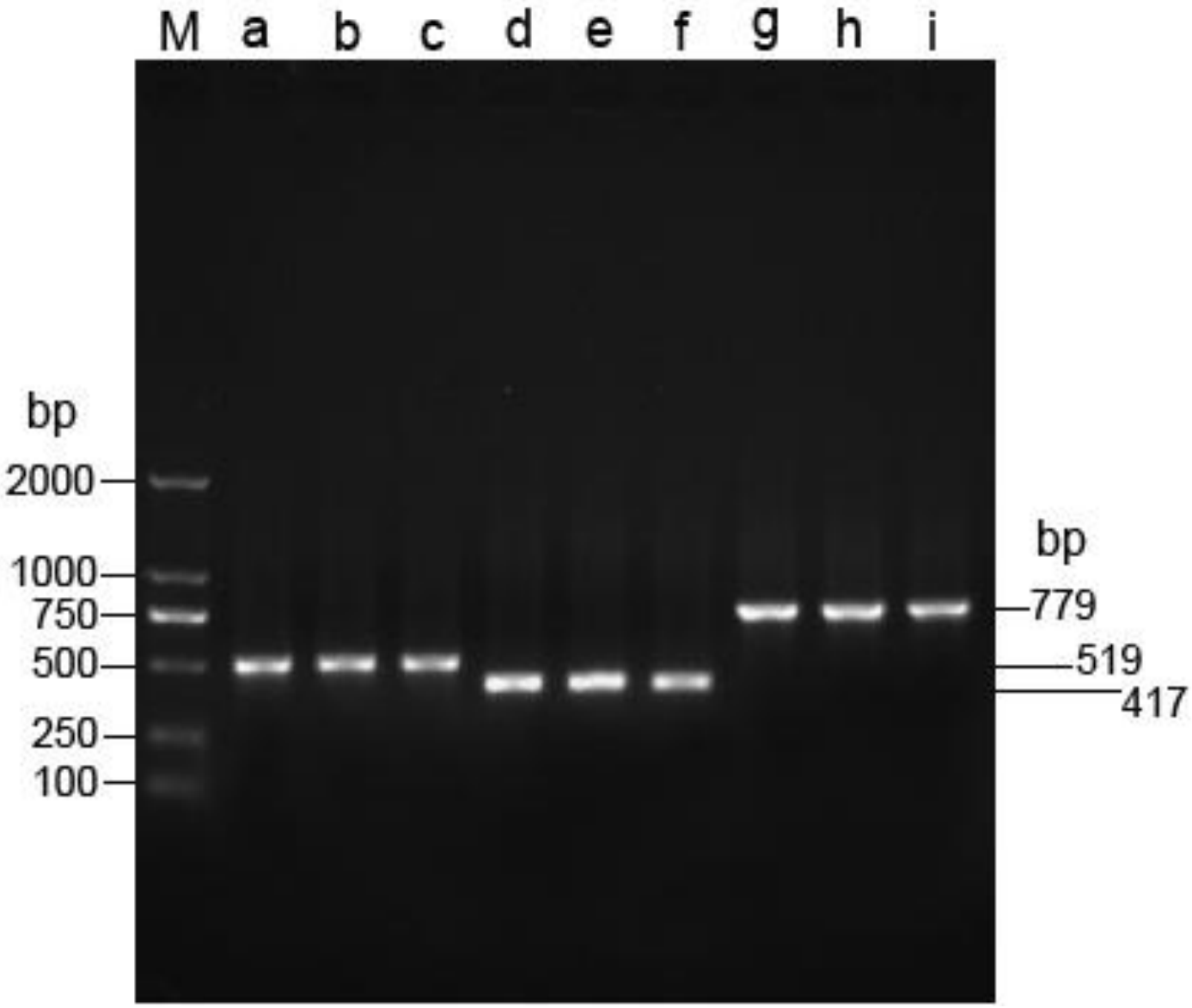

Figure 5. Agarose gel electrophoresis of PCR products of *lolB* (lanes a to c), *rtxA* (lanes d to f) and *toxR* (lanes g to i). Lanes a, d and g, NQ1; lanes b, e and h, NQ2; lanes c, f and i, NQ3; lanes M, molecular mass markers (DL 2000).

ceftriaxone, cephradine, azithromycin, lomefloxacin, levofloxacin, enoxacin, norfloxacin, neomycin, streptomycin, doxycyline, tetracycline, and minomycin,respectively. In our study, the isolates showed variable sensitivity and resistance to different agents of the same antimicrobial agent class. For example, the isolates were sensitive to carbenicillin and azlocillin, but resistant to penicillin G, ampicillin, oxacillin and amoxicillin (Table 3). Some reasons might take account for these differences. First, some bacteria exhibit intrinsic resistance to some antimicrobial agents. Second, long-term use of one agent in aquaculture may also lead to some bacterial's resistance to it. These results suggest that some antimicrobials (carbenicillin, azlocillin, cefoperazone, ceftazidime, ceftriaxone, cephradine, azithromycin, lomefloxacin, levofloxacin, enoxacin, norfloxacin, neomycin, streptomycin, doxycyline, tetrecycline and minomycin) could be employed to potentially prevent outbreaks of disease caused by *V. cholerae* in loach aqualculture.

ACKNOWLEGEMENTS

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Table 3. Name and type of discs and antimicrobial sensitivity of pathogenic *V. cholerae.*

Groups	Chemicals	Disc content (µg)	Sensitivity*
Penicillins	Penicillin G	10	R
	Ampicillin	10	R
	Carbenicillin	100	S
	Oxacillin	1	R
	Azlocillin	75	S
	Amoxicillin	10	R
Cephalosporins	Cefazolin	30	I
	Cefoperazone	30	S
	Ceftazidime	30	S
	Ceftriaxone	30	S
	Cephradine	30	S
Macrolides	Midecamycin	30	I
	Azithromycin	15	S
	Clarithromycin	15	R
	Clindamycin	2	R
	Erythromycin	15	R
Quinolones	Lomefloxacin	10	S
	Levofloxacin	5	S
	Enoxacin	10	S
	Norfloxacin	10	S
Aminoglycosides	Gentamycin	120	I
	Neomycin	30	S
	Vancomycin	30	I
	Streptomycin	10	S
Tetracyclines	Doxycyline	30	S
	Tetraecycline	30	S
	Minomycin	30	S
Sulfonamides	Trimethoprim/ Sulfamethoxazole	1.25/23.75	R

Science Fund of Jiangsu Province (BK2009163).

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