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RESEARCH ARTICLE

Characterization of *Vogesella perlucida* associated with ascites of diseased *Clarias gariepinus* (Burchell) with and ruptured intestine syndrome

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Abstract. The incidence of diseases has been increasing along with the global production of catfish in aquaculture. *Vogesella perlucida* has not been recognized as a fish pathogen. This study characterized the *V. perlucida* associated with diseased catfish, *Clarias gariepinus* (Burchell), with phenotypic and molecular methods. The ascites of diseased larvae had *Aeromonas* spp. and *Pseudomonas* spp. The translucent strain CGB1, phenotypically identified as *Pseudomonas* sp., was confirmed as *V. perlucida* with 99% nucleotide similarity. This strain was negative for gelatinase, amylase, lipase, DNAse, and hemolytic activities. No signs of external infection, behavioral abnormalities, or mortality were observed in catfish larvae when immersed challenged at 6.30 × 10^7 *V. perlucida* cells mL^{-1}. The results of preliminary in-vitro virulence and immersion assays suggested that *V. perlucida* had no pathogenic potential to cause disease in catfish. Although the results provided the basic phenotypic and genotypic data on fish-borne *V. perlucida* (NCBI accession number KJ522788), establishing a host-pathogen relationship at the molecular level was needed to ascertain what kind of relationship this bacterium had with the diseased catfish. Nevertheless, reports on the increasing incidences of *Vogesella* spp. and *V. perlucida* with diseased fish in recent years is cause for concern.

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Introduction

The genus *Vogesella* (class Betaproteobacteria; family Neisseriaceae) was named in honor of Otto Voges, who first isolated and described this blue-pigmented eubacterium in 1893 (Grimes et al. 1997). Important species from the genus *Vogesella* include *V. indigofera,* a blue-pigment producing bacterium (Grimes et al. 1997); *V. perlucida,* a non-pigmented bacterium isolated from spring water (Chou et al. 2008); *V. lacus,* a non-pigmented bacterium isolated from a soft-shell turtle culture pond (Chou et al. 2009); *V. mureinivorans,* a peptidoglycan-degrading non-pigmented bacterium from lake water (Jřrgensen et al. 2010); *V. alkaliphila*, a blue-pigment producing bacterium isolated from alkaline soil (Subhash et al. 2013); and *V. fluminis* isolated from a freshwater river (Sheu et al. 2013). Characteristically, they are short, Gram-negative, rod shaped, non-sporulating bacteria that are motile with a single polar flagellum. They

are most commonly reported from tropical freshwater ecosystems. They grow optimally in aerobic conditions in tryptic soy broth (TSB) from 5–37°C, pH 6–9, at 20–30°C (Chou et al. 2008, 2009). *V. perlucida* (type strain DS-28^T) was first isolated from a spring water sample from Tainan County, Taiwan (Chou et al. 2008). A review of the literature revealed the association of *V. perlucida* with the bacterioplankton community in the aquaria water column of the ornamental fishes *Pterophyllum scalare* and *Archocentrus nigrofasciatus* (Vlahos et al. 2013), the kidney of diseased Nile tilapia, *Oreochromis niloticus* (Soto-Rodriguez et al. 2013), the gill tissue of *Catla catla* (CIFRI 2018), skin nodules and livers of diseased *Betta splendens* (Dong et al. 2018), and diseased cage cultured *O. niloticus* (Preena et al. 2020).

Global fish production peaked at about 178 million tons in 2018, with aquaculture representing 46% of total fish production (FAO 2020). The growth from aquaculture in India has been stupendous at 0.37 MT in 1980–81 to 7.066 MT in 2018 (DOF GOI 2020). Catfish are currently produced worldwide in various production systems ranging from very low-yield extensive systems to high-yield intensive systems. The global production of catfish has increased from about 0.32 million tons in 1992 to about 4.155 million tons in 2014. The USA is still the world's largest producer of catfish (FAO 2020). In India, catfish production has been increasing over the years thanks to high economic returns with low inputs. India produced about 212.46 thousand tons of catfish in 2010. Current production reaches 10 ton/ha, but the Government of India is aiming to increase unit area production through diversification backed by new policies and state initiatives (DOF GOI 2020). With the intensification of aquaculture practices, there have been frequent occurrences of infectious diseases in Indian catfish aquaculture (Abraham et al. 2015, 2016, 2018, Paul et al. 2015). This communication reports on phenotypic, phylogenetic, and pathogenic characterizations of a hitherto unfamiliar *Vogesella perlucida* from the ascites of *C. gariepinus* larva with dropsy and ruptured intestine syndrome.

Materials and methods

Sampling and bacteriology

During routine fish disease surveillance, African catfish, *C. gariepinus*, larvae (≤ 1.0 g; 2.0–2.5 cm) aged 10–12 days with typical symptoms of dropsy in a hatchery located in Ramchandrapur (Lat 22°53'21" N; Long 88°28'13'' E), North 24 Parganas district, West Bengal, India were examined as per Heil (2009). At the site, behavioral abnormalities and gross and clinical signs of diseased *C. gariepinus* larvae were recorded. Morbid *C. gariepinus* larvae with typical disease symptoms were brought to the laboratory in oxygen-filled polythene bags for bacteriological analysis. Before this, the catfish larvae with dropsy (Fig. S1) were first rinsed in sterile saline, wiped with sterile paper towels, and the swollen abdomens of the larvae were punctured with sterile needles. Inocula from the ascites (n = 5) were collected aseptically separately, streaked onto brain heart infusion agar (BHIA), glutamate starch phenol red agar (GSPA), *Aeromonas* isolation agar (HiMedia 2009), and *Edwardsiella ictaluri* agar (EIA) (Shotts and Waltman II 1990) plates, and incubated at 30 ± 2°C for 24–48 h. Based on the dominance and definite colony morphology, representative colonies were chosen, purified on BHIA, and maintained on BHIA slants at room temperature and also as a glycerol stock at -20°C.

Phenotypic characterization of bacterial isolates

A series of biochemical reactions were performed to identify a few bacterial strains (n = 6), as shown in Table 1, and to further characterize certain virulence factors (Collins et al. 2004). The phenotypic identification of *Aeromonas* spp. and *Pseudomonas* spp. was done conventionally (Collins et al. 2004; Austin and Austin 2012). The translucent strain CGB1 was identified with an automated bacterial identification system (VITEK-2 Compact, bioMérieux, France).

Table 1
General and biochemical characteristics of the bacterial flora associated with ascites of *Clarias gariepinus* larvae by conventional tests

General and biochemical characteristics	*Aeromonas sobria*-like	*Aeromonas trota*-like	*Aeromonas veronii* biovar *sobria*-like	*Pseudomonas alcaligenes*-like	*Pseudomonas* sp*.	*Pseudomonas pseudomallei*-like
Growth medium	AIA	AIA	GSPA	GSPA	BHIA	EIA
Colony color	Green with a dark center and light green periphery	Green with black center	Light yellow	Pink	Translucent	Dark green center with a translucent periphery
Colony character	Round	Glossy	Flat, round	Flat, round	Round	Round
Colony diameter, mm	2.0	1.5–2.0	2.0	2.0	1.5	2.0
Gram reaction	-	-	-	-	-	-
Morphology	R	R	R	R	R	R
Oxidase	+	+	+	+	+	+
O/F reaction	+/+	+/+	+/+	-/-	+/-	+/-
Motility	+	+	+	+	+	+
Gas from glucose	+	+	+	ND	-	-
Indole	+	+	+	-	+	-
VP reaction	-	-	+	-	-	-
Citrate utilization	+	+	-	+	-	+
Starch hydrolysis	+	-	-	-	-	+
Esculin hydrolysis	-	-	-	-	-	-
Arabinose utilization	-	-	-	-	-	+
Cellobiose utilization	+	+	-	-	-	+
Sorbitol	-	-	-	-	-	+
Lysine decarboxylase	+	+	+	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-
Arginine dihydrolase	-	+	+	+	-	-
Diffusible pigment	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+

BHIA – Brain heart infusion agar; GSPA – Glutamate starch phenol red agar; AIA – *Aeromonas* isolation agar; EIA – *Edwardsiella ictaluri* agar; * – identified as *V. perlucida* based on molecular characterization; ND – not done.

PCR amplification of 16S rRNA gene of the bacterial strain CGB1

Genotypic characterization of the bacterial strain CGB1, grown overnight in TSB, was done by 16S rRNA gene amplification and sequencing. Genomic DNA was extracted using a genomic DNA isolation kit (Macherey-Nagel, Germany) as per the manufacturer's protocol. The 16S rRNA gene was amplified in a Master cycler Pro S system (Eppendorf, Germany) using universal primers 8F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'-ACGGCT ACCTTGTTACGACTT-3' of an amplification size of 1400 bp (Eden et al. 1991). The reaction mixture (25 µL) comprised 12.5 µL 2X PCR TaqMixture, 1.0 µL forward primer 8F (10 pMol μL^{-1}), 1.0 µL reverse primer 1492R (10 pMol μL^{-1}), 1.0 µL DNA template, and 9.5 µL molecular biology grade water. The PCR components were mixed and spun briefly. The amplification was done by initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 45 sec, primer annealing at 55°C for 1 min, and extension at 72°C for 1 min. The final extension was at 72°C for 10 min. The PCR product was electrophoresed in 1.5% agarose gel containing 0.5 µg mL^{-1} ethidium bromide in 1X Tris-acetate-EDTA buffer.

DNA sequencing and phylogenetic analyses

The PCR amplicon was sequenced at the Genomics Division, Xcelris Labs Limited, Ahmadabad, India.

The amplicon was first purified using the EXO-SAP treatment and the concentration was determined. The purified DNA was subjected to automated DNA sequencing on an ABI 3730 × l Genetic Analyzer (Applied Biosystems, USA). Sequencing was carried out using a BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, USA) following the manufacturers' instructions. Electrophoresis and data analysis were conducted on the ABI 3730 × l Genetic Analyzer. Sequence data analysis, multiple alignments, and genetic distance analyses were done with the Kimura 2-parameter model, and evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011). A preliminary phylogenetic analysis was conducted using the basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI). The final phylogenetic analyses were performed on a selection of 16S rDNA sequences that comprised the new sequence and 25 additional sequences from the NCBI Genbank database using BLAST (http://blast.ncbi.nlm.nih.gov). The evolutionary history was inferred using the neighbor-joining (NJ) method in MEGA5.

Histopathology

Whole larvae were first fixed in alcoholic Bouin's fixative for 48-h and then preserved overnight in 70% ethyl alcohol. Sample processing and the histopathological analysis were performed as per Roberts (2012).

Pathogenicity of *V. perlucida* on *C. gariepinus* larvae by immersion assay

C. gariepinus larvae (≤ 1.0 g; 2.0-2.5 cm) were procured from Naihati, Bodtalla fish market, North 24 Parganas district, West Bengal, India, and brought to the laboratory in oxygen-filled polythene bags. The larvae (n = 200) were first disinfected with 5-ppm potassium permanganate for 5 min, stocked in 500 L capacity fiberglass reinforced plastic tanks containing 300 L clean bore-well water, and acclimatized for about 10 days. They were fed with overnight depurated *Tubifex* sp. in running water at the rate of 2% body weight. The accumulated waste and feces were removed once every three days and 50% of the water was exchanged.

The acclimatized catfish were checked for gross and external signs of diseases visually and systemic infection as described in Abraham et al. (2016) on BHIA, GSPA, and EIA before the challenge. A *V. perlucida* CGB1 cell suspension was prepared as described in Abraham et al. (2016) and the number of bacterial cells in saline suspension was determined by spread plating on BHIA. The pathogenicity of *V. perlucida* was tested by immersion at a predetermined dose in triplicate. Six glass aquaria (45 × 30 × 30 cm) were filled with 15 L of clean bore-well water each and conditioned for three days. Twenty healthy fish were selected, released into the experimental glass aquaria, and acclimatized for three days with continuous aeration. All fish were fed *Tubifex* sp. and maintained under optimal conditions. Two mL each of the *V. perlucida* cell suspension containing 8.40×10^{10} cells mL^{-1} was added to three glass aquaria to a concentration of about 6.30×10^{7} cells mL^{-1}. The control group received no bacterial inoculum. The challenged and control groups were maintained in the respective aquaria for 20 days. Behavioral abnormalities, external signs of infection, and mortality, if any, were recorded daily.

Results

The gross and clinical signs observed in the diseased larvae included lethargy, red spots on the head, dark coloration, fin rot, tail rot, focal cutaneous and abdominal hemorrhages, dropsy (Fig. S1), fluid accumulation in the abdomen (ascites), discoloration of internal organs, inflammation of internal organs and intestine, visceral hemorrhages, and gas accumulation. A foul smell was noted in a few cases. Mortality was about 80% within a week and about 35-40% of the dead larvae had burst bellies. From the ascites, *Aeromonas sobria*-like, *A. trota*-like, *A. veronii* biovar *sobria*-like, *Pseudomonas alcaligenes*-like, *P.*

Table 2
Biochemical characteristics of *Vogesella perlucida* strain CGB1* and its comparison with *V. perlucida* strain DS-28$^{T\#}$

Characteristics	CGB1	DS-28^{T}	Characteristics	CGB1	DS-28^{T}
D-Glucose (dGLU)	+	+	Ala-Phe-Pro-arylamidase (APPA)	-	ND
Saccharose/Sucrose (SAC)	+	+	L Pyrrolydonyl-arylamidase (PyrA)	-	ND
D-Cellobiose (dCEL)	-	-	Beta-N-Acetyl-glucosaminidase (BNAG)	-	-
Adonitol (ADO)	-	-	Glutamyl arylamidase pNA (AGLTp)	+	ND
L-Arabitol (IARL)	-	ND	Gamma-glutamyl transferase (GGT)	-	ND
D-Mannitol (dMAN)	+	+	Beta-alanine arylamidase pNA (BAlap)	-	ND
D-Sorbitol (dSOR)	-	-	L-Proline arylamidase (ProA)	-	ND
D-Maltose (dMAL)	+	+	Tyrosine arylamidase (TyrA)	+	ND
D-Mannose (dMNE)	-	-	Beta-N-acetyl-galactosaminidase (NAGA)	-	ND
D-Tagatose (dTAG)	-	ND	Glycine arylamidase (GlyA)	-	ND
D-Trehalose (dTRE)	+	-	Glu-Gly-Arg-arylamidase (GGAA)	-	ND
H2S production (H2S)	-	-	Ellman (ELLM)	-	ND
Lysine decarboxylase (LDC)	-	-	L-Lactate assimilation (ILATa)	-	ND
Ornithine decarboxylase (ODC)	-	-	L-Histidine assimilation (IHISa)	-	ND
L-Malate assimilation (IMLTa)	-	+	5-Keto D-gluconate (5KG)	-	ND
Citrate (sodium) (CIT)	-	-	Palatinose (PLE)	-	ND
Urease (URE)	-	-	Malonate (MNT)	-	ND
Fermentation/ glucose (OFF)	-	-	L-Lactate alkalinization (ILATk)	+	ND
Phosphatase (PHOS)	-	ND	Succinate alkalinization (SUCT)	+	ND
Beta-glucoronidase (BGUR)	-	-	Alpha-galactosidase (AGAL)	-	-
Lipase (LIP)	-	+	Beta-glucosidase (BGLU)	-	-
Alpha-glucosidase (AGLU)	-	+	Coumarate (CMT)	-	ND
Beta-xylosidase (BXYL)	-	ND	O/129 Resistance (O129R)	+	ND
Beta-galactosidase (BGAL)	-	-			

*– VITEK-2 Compact system (bioMérieux, France) identified the strain CGB1 as *Sphingomonas paucimobilis,* as it did not have the database on *Vogesella perlucida*; ND – no data; # – Chou et al. (2008).

pseudomallei-like and *Pseudomonas* sp. were isolated (Table 1). The presumptive *Pseudomonas* sp. CGB1 was identified as *Sphingomonas paucimobilis* with 86% probability by the VITEK-2 system (Table 2). No typical colonies of *Edwardsiella tarda* could be detected on EIA.

The universal prokaryotic primers 8F and 1492R were successfully amplified to the ≈1500 bp sequence of the 16S rDNA gene of the CGB1 strain. The consensus sequence was a continuous stretch of 1411 bp. The strain CGB1 formed a distinct subline within the genus *Vogesella* of the class Betaproteobacteria. It formed a monophyletic group with other strains of *V. perlucida* with 99% nucleotide similarity, except *V. perlucida* (NCBI accession number KF530800), which exhibited only 94% nucleotide similarity. The lower sequence similarities (83–95%) were found with other representative members selected for the present study. In the NJ tree, it was closely related to *V. perlucida* (NCBI accession number KC706673, 99%) with low node support (Fig. 1). The nucleotide sequence of the present study was deposited in the NCBI GenBank database under accession number KJ522788. Of the 11 submissions on the 16S rDNA gene sequences of *V. perlucida* from the normal and diseased fish available in the NCBI GenBank database, six were from diseased fish (Table 3). *V. perlucida* CGB1 was negative for the production of protease, caseinase, gelatinase, amylase, DNAse, hemolysin, and chitinase (Table 4). When challenged by immersion at 6.30×10^{7} cells mL^{-1}, it caused no signs of abnormalities, external infection, or mortality during the experimental period. The histological section of the

Table 3
16S rRNA sequences of *Vogesella perlucida* strains of normal and diseased fish available in the NCBI GenBank database

Strain number	GenBank Accession number	Sequence size, bp	Host/ Source/Site of infection	Origin
C6 11m	KC706673	1516	Kidney of *Oreochromis niloticus*	Sonalona, Mexico
C10 4m	KC706672	1526	Kidney of *O. niloticus* with exophthalmia	Sonalona, Mexico
S4	KM268941	1068	Eel	Xiamen, China
M8	KM268937	1027	Eel	Xiamen, China
M5	KM268934	1075	Eel	Xiamen, China
P4	KF530800	598	Fish	Machilipatnam, India
CIVPCG-1	MG757246	1407	Gill tissue of Catla catla	Kolkata, India
BN 2063	MG438520	1499	Skin nodule of *Betta splendens* with SNS	Bangkok, Thailand
BN 2066	MG438523	1499	Liver of *Betta splendens* with BBS	Bangkok, Thailand
TL1	MN493068	1414	Diseased *Oreochromis niloticus*	Cochin, India
CGB1	KJ522788	1411	Ascites of *Clarias gariepinus* with RIS	Kolkata, India

RIS – ruptured intestine syndrome; SNS – skin nodule syndrome; BBS – big belly syndrome.

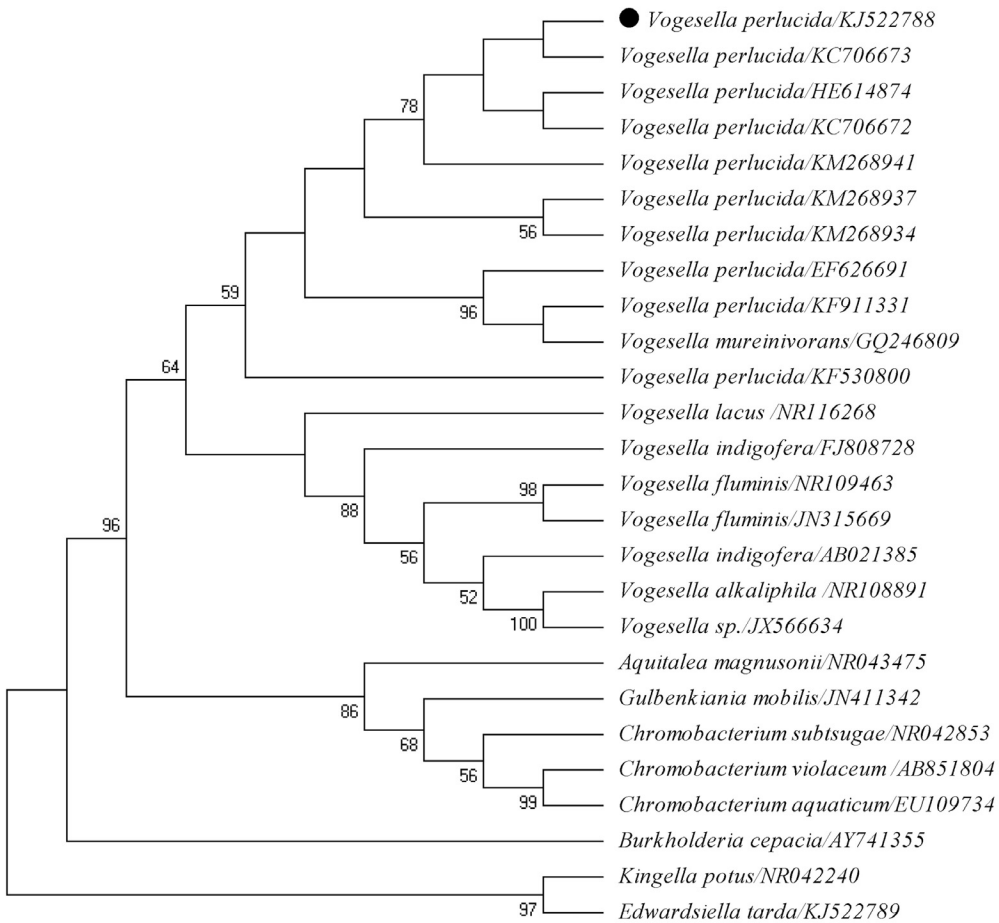

Figure 1. Phylogenetic tree generated by neighbor-joining of the 16S rRNA gene sequences of *Vogesella perlucida* CGB1 (Accession number KJ522788), closely related species, and other bacterial representatives available in the NCBI GenBank database. Bootstrap confidence values are shown at nodes (1000 replications).

Table 4
Virulence of *Vogesella perlucida* strains

Virulence factors/test	*V. perlucida* DS-28^T (Chou et al. 2008)	*V. perlucida* C10 4 m (Soto-Rodriguez et al. 2013)	*V. perlucida* CGB1 (Present study)
Gelatinase	-	-	-
Caseinase	-	-	-
Lipase	+*	-	-
Amylase	-	ND	-
Urease	-	ND	-
Chitinase	-	ND	ND
DNAse	-	-	-
Haemolysin	-	-	-
Esculin hydrolysis	-	ND	-
Hydrogen sulphide production	-	ND	-
Cytotoxic assay on HeLa cell lines	ND	-	ND

* – C14 corn oil; ND – not done. No signs of behavioral abnormalities, external infection, or mortality were observed in catfish fry when immersed in a medium containing 6.30×10^7 *V. perlucida* cells mL^{-1}.

internal organs of naturally diseased *C. gariepinus* showed degenerative changes and ruptured intestine (Fig. 2).

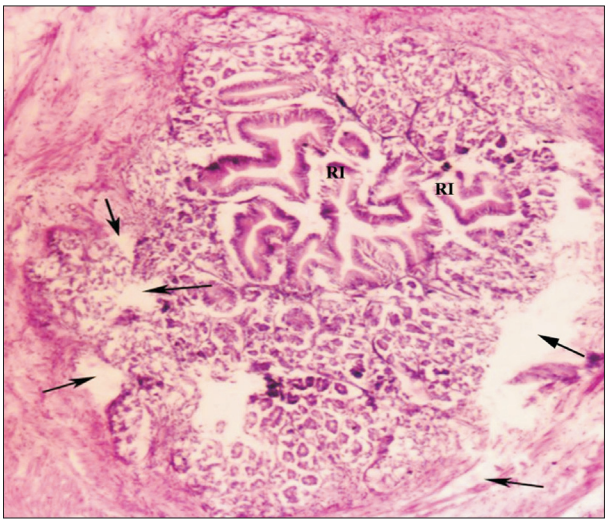

Figure 2. Photomicrography showing the internal organs of diseased *Clarias gariepinus* with degenerative changes (→) and ruptured intestine (RI), X200 H&E staining.

Discussion

It was observed in the surveyed hatchery that the *C. gariepinus* larvae were stocked at high densities (≥ 25 larvae L^{-1}) in cement tanks with a water depth of about 20 cm. Water circulation, aeration, feed, and health management measures were inadequate. The larvae were often fed excessively due to their voracious feeding behavior. About 80% of the catfish larvae succumbed to disease within a week. The dead larvae had burst belly, a condition similar to ruptured intestine syndrome (RIS) or open belly syndrome, which starts as a local inflammation of the ileum or the rectum resulting in a perforation of the intestine (Boon et al. 1987). According to Boon et al. (1987), high feeding levels resulted in a higher percentage of RIS possibly due to the autodigestion of the gut mucosa. Histologically, it was confirmed in this study that the affected larvae had ruptured intestines with marked degenerative as well as inflammatory changes in the internal organs. The isolation of *Aeromonas sobria*, *A. trota*, *A. veronii* biovar *sobria*, *Pseudomonas alcaligenes*, *P. pseudomallei*, and *Pseudomonas* sp. (*V. perlucida*) from the ascites of diseased catfish larvae possibly indicated the involvement of bacteria originating from the gut in causing peritoneum inflammation. The spread of inflammation to other parts of the gut and of the abdominal muscles prompted exudate production in the abdominal cavity that lead to dropsy. The degeneration and finally the maceration of the abdominal wall might have resulted in bursting belly with the fluid leaving the abdominal cavity, and, thus, the "open belly" dead fish.

The association of *Aeromonas* spp. and *Pseudomonas* spp. was reported in diseased catfish with infectious dropsy (Edun 2007) and also in the present study. Since the majority of the bacterial species identified are known as opportunistic fish pathogens (Austin and Austin 2012), attention was focused on the translucent strain CGB1, which was presumptively identified as *Pseudomonas* sp. VITEK-2 identified strain CGB1 as *Sphingomonas paucimobilis* with low probability, because the system did not include the database on *V. perlucida*. VITEK-2 data were, therefore, used for the phenotypic characterization of strain CGB1. Biochemically, the present strain and *V. perlucida* DS-28^T (Chou et al. 2008) were almost similar and comparable, except for trehalose, lipase, L-malate assimilation, and alpha-glucosidase. The genotypic characters and phylogenetic analyses, thus, identified the bacterial strain CGB1 from the ascites of *C. gariepinus* larva as *V. perlucida*.

The pathology of *Vogesella* spp. in humans and animals is largely unclear. Van Belkum (2009) categorized *Vogesella* as risk category 1. However, recently, Yu et al. (2020) reported the first case of a rare *V. perlucida*-induced bacteremia in an advanced-age patient with many basic diseases. It is important to mention here that of the 11 NCBI GenBank submissions on fish-borne *V. perlucida* 16S rDNA gene sequences, six were from diseased fish. Though the isolation and identification of *V. perlucida* in the kidney of exophthalmic *O. niloticus* farmed in floating cages from Mexico (Soto-Rodriguez et al. 2013), liver and skin nodules of diseased *B. splendens* from Thailand (Dong et al. 2018), and diseased cage cultured *O. niloticus* from India (Preena et al. 2020) revealed its association with diseased fish, none proved its pathogenic potential on fish. The experiments by Soto-Rodriguez et al. (2013) with extracellular products of the *V. perlucida* strain C10 4 m did not display any cytotoxic effects on HeLa cell monolayers at 12 h post-inoculation. The previously described *V. perlucida* strain DS-28^T was reportedly negative for the production of protease, caseinase, gelatinase, amylase, DNAse, hemolysin, and chitinase and positive for lipase on C14 corn oil (Chou et al. 2008) as also was the present strain. *V. perlucida* CGB1 did not induce hemolysis on sheep blood agar. The immersion assay at 6.30×10^7 cells mL^{-1} proved its inability to cause disease or mortality and, thus, provided supportive evidence to van Belkum (2009). Additional studies are, however, required using other challenge modes to elicit its pathology. To date, *V. perlucida* is considered an environmental bacterium and has not been recognized as a fish pathogen. The results of the earlier studies (Chou et al. 2008, Soto-Rodriguez et al. 2013, Dong et al. 2018, Preena et al. 2020) and the preliminary in-vitro and the immersion assay results of the present study, demonstrated that *V. perlucida* CGB1 is an avirulent strain and has no pathogenic potential to cause disease in catfish larvae. Further, the association of *V. indigofera* in the pyloric ceca of wild fish, which had leeches attached to them (Goldschmidt-Clermont et al. 2008), and *Vogesella* spp. in the microbial community of the piscicolid leech *Myzobdella lugubris,* heavily leech parasitized channel catfish *Ictalurus punctatus*, and freshwater drum *Aplodinotus grunniens* tissue homogenates (Schulz and Faisal 2010), the gastrointestinal tract of carp *Cyprinus carpio* L. (van Kessel et al. 2011), gastrointestinal microbiota of seabirds (Dewar 2012), the intestinal microbiota of cultured rainbow trout *Oncorhynchus mykiss* (Wong et al. 2013), finfish from India (NCBI GenBank accession number KF530800), eel from China (KM268934; KM268937 and KM268941), and gut microbiota of cotton leafworm, *Spodoptera littoralis* (Shao et al. 2014) suggested that the *Vogesella* spp., including *V. perlucida*, is normal flora of fish and other vertebrate and invertebrate organisms.

Conclusion

From our observations on the isolation of *V. perlucida* from diseased fish in this study and the association of *V. perlucida*-like organisms in other case studies with diseased carps and catfish cultured in West Bengal, India, we speculate that *V. perlucida* along with other known fish pathogens of cultured

fish might play an important synergistic role in disease progression. It also appears from the observations above that *Vogesella* spp. are more commonly associated with the gut and might have entered the ascites of catfish larvae through ruptured intestine. Nevertheless, the emergence of reports on the increasing incidences of *Vogesella* spp. and the association of *V. perlucida* with diseased fish, in recent years, is cause for concern.

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Author contribution. T.J.A. designed the experiment, procured the experimental fish, supervised the research work, and checked the manuscript. H.A. and P.P. collected the field samples and executed the bacteriological experiments. A.P. and S.B. were involved in the molecular characterization and histopathology, respectively. All authors approved the manuscript for publication.

Conflict of interest The authors declare no conflicts of interest.

Ethical approval and consent to participate. This article does not contain any studies with human participants performed by any of the authors. All applicable guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA 2018), Government of India, New Delhi were followed.

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Figure S1. *Clarias gariepinus* larva with inflamed internal organs, abdominal hemorrhage, and dropsy.