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Ornamental fish aquaria as a reservoir of unusual predatory bacteria

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ABSTRACT

Aquaria that host ornamental fish may support a vast array of microorganisms, but to date, little information is available on these microbial ecosystems. In the present study, a polyphasic approach based on conventional and culture-independent strategies was used to evaluate the microbial community in the filter and in the gravel of a home aquarium harbouring *Pristella maxillaris*. The presence of predatory bacteria was also assessed, and four predatory bacteria could not be identified as belonging to any known species within the genus *Bdellovibrio*. Strains differed in terms of prey preference but shared the same RAPD profiles. A total of 62 cultures were isolated and identified by 16S rDNA sequencing. *Aeromonas hydrophila* accounted for 82% and 50% of the isolates in the filter and gravel, respectively. However, recently isolated *Pseudomonas* species such as *Pseudomonas ullengensis*, *Pseudomonas lalkuanensis*, and *Aquipseudomonas alcaligenes* were also retrieved. According to high-throughput sequencing, in gravel, eubacteria accounted for 85.42%, while Archaea accounted for 14.57%. In the filter, eubacteria dominated (98.25%). Archaea were almost entirely represented by ammonium oxidizers, *Nitrosopumilaceae* (14.30%). Conversely, in the filter, ammonium oxidation was apparently carried out by *Nitrosomonadaceae* (19.52%). Home aquaria appeared to be complex ecosystems and sources of unusual predatory bacteria.

1. Introduction

Keeping ornamental fish as pets in an aquarium is increasingly popular in households (Perry, 2023). When the coronavirus pandemic forced people to stay at home, the popularity of aquarium fish as a hobby skyrocketed (Khalifa et al., 2021). Keeping fish has been linked to several health benefits for humans, including lowering blood pressure, reducing the risk of cardiovascular disease and mortality, decreasing loneliness, and providing emotional support during mental health challenges (Clements et al., 2019).

The water in aquatic systems that support animal hosts a vast array of microorganisms. A single millilitre of surface seawater may contain a million bacterial cells and 10 million viruses (Van Bonn et al., 2015).

To date, only a few studies have characterized the overall microbial population structures associated with animal species or water in aquaria. By a culturomics approach, Raja et al. (2006) detected high diversity coupled to an abundance of *Vibrio* and other potential human pathogens in aquarium water. Using culture-independent techniques, Sugita et al. (2005) characterized the microbial communities associated with filter materials in closed, recirculating systems for carp and goldfish; Smith et al. (2012) identified potential pathogens for fish and humans by analysing the aquarium water supporting goldfish and Chinese algae eaters. Van Bonn et al. (2015) explored the bacterial community composition of an established teleost fish system, demonstrating that the bacterial community diversity and evenness increased significantly after a 90% water replacement.

From this perspective, aquaria have been suggested as an ideal model system for revealing organism–environment interactions (Kim et al., 2017). To date, little is known about the microbiome of aquariums housing ornamental fish other than goldfish. Furthermore, only one study focused on the ecosystem of householder aquariums as a reservoir of aquatic BALOs (Williams et al., 1987). BALOs (*Bdellovibrio* and like organisms) are a group of relatively small Gram-negative bacteria characterized by an obligate predatory feeding behaviour on other Gram-negative bacteria (Mookherjee and Jurkevitch, 2022). Pérez et al. (2016) reviewed the hunting strategies of predators of the order *Bdellovibrionales,* which physically attach to prey cells with flagella-based motility and penetrate the periplasm of the prey cells, and the order *Myxococcales*, which are known for a “group attack” with gliding motility, the secretion of lytic enzymes, and the release of antibiotics. Through these mechanisms, BALOs can modify the organization of microbial communities, regulate vulnerable bacterial populations, and

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support biogeochemical cycling. BALOs are environmental organisms ubiquitous in fresh and salt waters, soils, sewage, and plant systems, as well as in animal bodies (Blaiotta et al., 2025).

With this goal, BALOs were searched in the water of a home aquarium hosting *Pristella* (*P.*) *maxillaris* and the aquatic plant *Anubias barteri* var. *nana*. This valuable ornamental fish, also known as the X-ray fish, has a huge market. Widely distributed and adaptable, *P. maxillaris* can be found in the Amazon and Orinoco basins, as well as in coastal rivers in the Guianas. Such warm water fish belong to the family Characidae and the order Characiformes (Bian et al., 2019). Additionally, both culture-dependent and independent methods were used to assess the microbiome of the filter and aquarium gravel.

2. Experimental procedures

2.1. Sampling

Water samples were collected from a tropical freshwater ornamental aquarium. The experimental tank - 60 cm × 30 cm × 35 cm (length × width × height) - represented an established ecosystem with a bottom substrate and plants (*Anubias barteri* var. *nana*). At the time of the experiment, the ten-year-old aquarium hosted eight adult *P. maxillaris* specimens. Both fish and plants were obtained from a local aquarium store. Fish were maintained under consistent conditions (24 ± 1°C; photoperiod of 8h-light, 16h-dark) and fed twice daily with artificial food.

Settings for the aquarium management were as follows: neon T8 15-Watt lighting, internal filter (filter wool, ceramic rings), monthly filter wool replacement, and water changes (20% of the total volume) every ten days using a mixture of tap (40%) and reverse osmosis (60%) water. Samples were taken 8 days after the water replacement and immediately before the filter cleaning.

The sample for the prey isolation was collected from the water (pH 8.08 ± 0.01; conductivity −63 ± 1.2 mV) column using a sterile glass bottle. The sample for isolating the BALOs was collected from the bottom of the tank, including water and gravel, using a sterile glass bottle. To favour the release of microorganisms, gravel samples were stirred (Heidolph Promax 2020) for 10 min. For microbial counts and metagenomic analysis, samples were also collected from the filter. Specifically, the filter wool was shifted, and a sterile pipette was used to collect the sample inside the ceramic rings. Samples were transferred to the laboratory under refrigerated conditions and analysed within one hour. pH and water conductivity were evaluated using a FiveEasy pH meter F20.

2.2. Prey isolation and identification

For the isolation of potential prey, water samples were serially diluted in sterile Ringer solution (Oxoid, Basingstoke, UK) and plated on MacConkey Agar No. 3 (Thermo Scientific™, Milan, Italy). Plates were incubated at 37°C overnight. Single colonies were purified by repeated streaking on the same medium and cultured on Luria Bertani Miller (LB) with 10 g l^{-1} of NaCl (Tryptone 10.0 g l^{-1}, Yeast extract 5.0 g l^{-1}, NaCl 10 g l^{-1}).

Cultures were checked for Gram reaction and catalase activity and identified by 16S rRNA sequencing by using primers and PCR conditions described by Weisburg et al. (1991). DNA was extracted through the protocol described by Wang et al. (1993). PCR amplicons were purified by QIAquick Gel Extraction Kit (Qiagen). Sequencing was carried out by Eurofins Genomics (Germany).

2.3. BALOs isolation

The enrichment protocol described by Jurkevitch (2006) was followed to isolate the marine BALOs. Two potential prey species were tested: *Aeromonas* (*A.*) *hydrophila* isolated during this study, plus one *Escherichia coli* strain (32) isolated from meat during a previous survey. Preys' overnight cultures in LB broth were centrifuged (6500 rpm for 15 min), and the cell pellets were resuspended in 100 ml of aquarium water previously filtered (Minisart 0.7 μm) to obtain a prey concentration around $10^9/10^{10}$ cell/ml. During incubation at 28°C under constant stirring (Orbital shaker 300 rpm), cultures were monitored daily by spectrophotometry (600 nm) and microscope observation (100x). Once the enrichment appeared clearer and small, fast-moving cells could be observed under the microscope, the enrichments were filtered (Minisart 0.45 μm) to remove small protozoa, and 10 ml of the filtrate was re-incubated with the same prey at a population level of about 5×10^9 cells/ml.

The double agar layer technique was used for isolation. The media used was Nutrient Broth diluted to 10% (DNB) and supplemented with $CaCl_2$ (3 mmol/l) and $MgCl_2{\cdot}6\ H_2O$ (2 mmol/l), pH 7.2. DNB for the bottom and top layers was prepared with 1.5% and 0.6% agar, respectively. Preys were centrifuged and resuspended in HM buffer [N-(2-Hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid) sodium salt] supplemented with 3 mmol/l $CaCl_2$ and 2 mmol/l $MgCl_2$ x 6 H_2O, pH 7.5, at a final concentration of 5×10^9 cells/ml.

For plating, 100 μl of the enrichment was serially diluted in HM buffer (900 μl) up to 10^{-6}. A 100 μl portion of each dilution was added to tubes containing 5 ml of molten DNB top agar and 400 μl of the bacterial prey suspension. Tubes were gently mixed and overlaid onto DNB bottom agar in petri dishes. Sealed plates were incubated at 28°C for 8 days and daily monitored for *Bdellovibrio*-like plaques. Lytic plaques were excised with a sterile cut tip and resuspended in 500 μl of HM buffer at pH 7.5 (Jurkevitch, 2006). After a few minutes of agitation, the liquid containing the released BALOs was serially diluted in HM buffer (up to 10^{-3}). Each dilution was then plated as described above to obtain pure plaques.

2.4. BALOs optimal temperature

To evaluate the optimal temperature range, a single pure plaque of the isolated BALO strains was excised and plated as described in paragraph 2.3. Prey used were *A. hydrophyla* and *E. coli* NCTC 10538. Plates were incubated at three different temperatures: 20°C, 28°C and 37°C. Lytic plaques were counted after 5 and 13 days of incubation.

2.5. BALOs identification and characterization

For DNA extraction, a single pure plaque was resuspended in an HM suspension of *A. hydrophyla* (5×10^9 cells/ml). Enrichments were incubated under constant stirring at room temperature. Upon clearance, the co-culture was filtered twice (Minisart 0.45 μm) and the pellet concentrated by centrifugation at 12.000 rcf for 10 min at 10°C. DNA was then extracted according to Wang et al. (1993). Strains were identified by 16S rRNA sequencing using primers and PCR conditions described by Weisburg et al. (1991) as described in paragraph 2.2. For phylogenetic analysis, 16S rRNA gene partial sequences were compared with those in the NCBI database. Multiple sequence alignments were performed using the Muscle command of MEGA11 software. A phylogenetic tree was constructed through the UPGMA tree (*p*-distance model) using MEGA 11 software with a bootstrap analysis of 1.000 replicates.

Strains were then biotyped by RAPD-PCR with primers M13 (Rossetti and Giraffa, 2005) and M13-R2 (Aymerich et al., 2003).

2.6. Aquarium microbial characterization by conventional counting methods

The gravel sample was stirred (Heidolph Promax 2020) to release the microorganisms. Gravel and filter samples were serially diluted and plated on Plate Count agar (PCA) after incubation at 30°C for 3 days. Enterobacteriaceae were monitored on Violet Red Bile Glucose agar (VRBGA) after incubation at 37°C for 24–48 h in microaerophilic

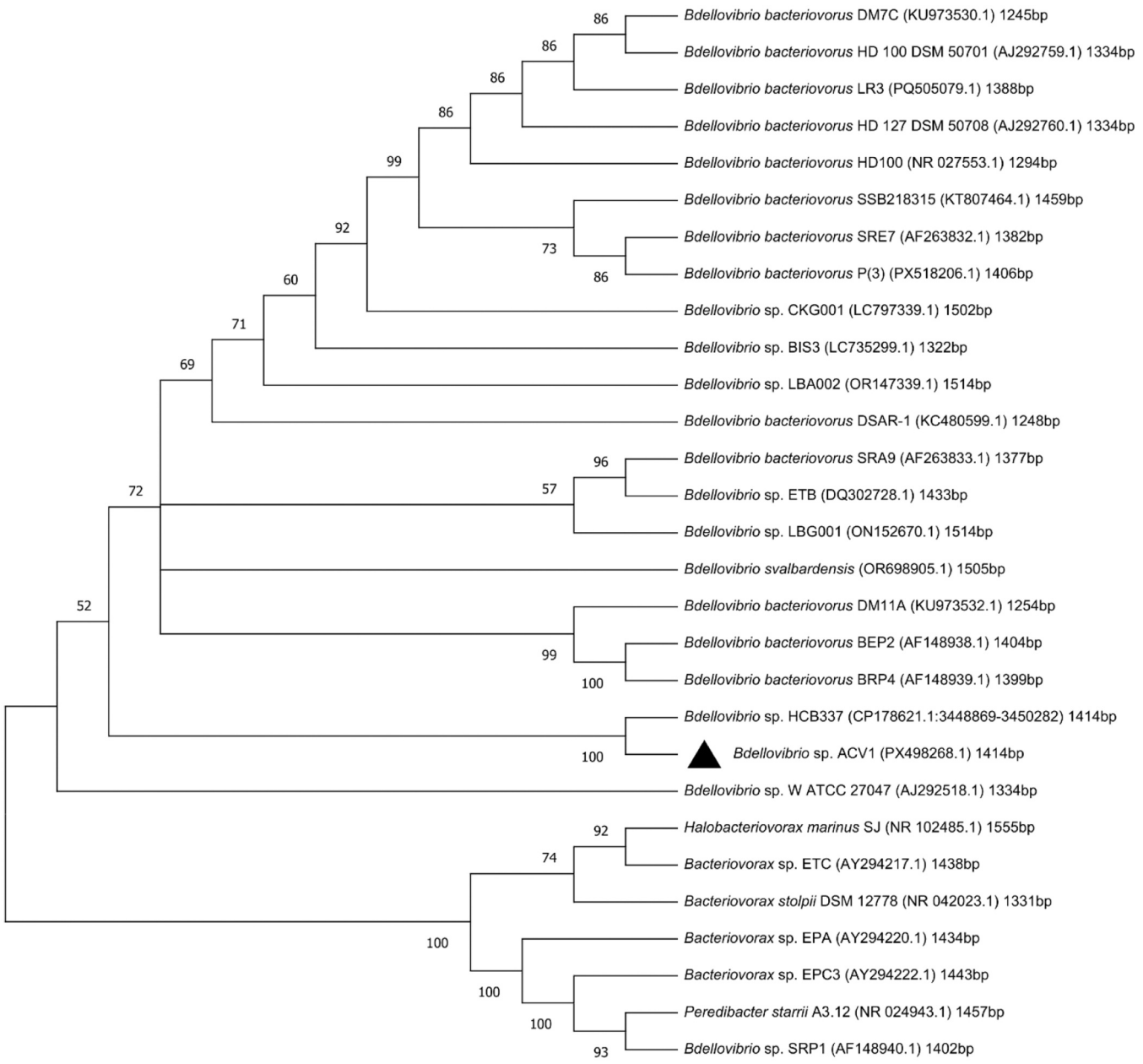

Fig. 1. Phylogenetic tree of partial 16S rRNA gene sequences of *Bdellovibrio sp*. ACV1. The UPGMA phylogenetic tree was constructed using MEGA 11 software with the p-distance method. The tree was generated using the bootstrap method, which was carried out 1000 times. *Bdellovibrio sp*. ACV1 is marked with a triangle.

conditions generated by the double-layer technique. Faecal coliforms and enterococci were assessed on Fecal Coliforms Agar Base (m-FC) and Slanetz & Bartley, in both cases after incubation at 37°C for 24–48 h. *Pseudomonadaceae* were counted on Pseudomonas Agar Base plus CFC supplement after incubation at 28°C for 48 h, Gram-negative bacteria on MacConkey agar after incubation at 37°C for 48 h, and lactic acid bacteria (LAB) on MRS after incubation at 30°C for 48 h. Yeasts and moulds were evaluated on Dichloran-Rose Bengal Chloramphenicol Agar (DRBC) after incubation at 28°C for 48 h.

At least three colonies were randomly picked from the countable plates of all media, except DRBC and MRS. Cultures were identified by 16S rRNA sequencing.

2.7. High-throughput sequencing analysis (HTS) of aquarium bacterial communities

Total DNA was extracted from the samples using the DNeasy® PowerSoil Pro Kits (Qiagen). Bacterial communities were assessed by HTS of the amplified V3–V4 regions within the 16S rRNA gene (~460 bp). PCR was carried out with primers (S-D-Bact−0341-b-S−17/ S-D Bact0785-a-A−21) connecting with barcodes (Aponte et al., 2022). PCR products with the proper size were selected by 2% agarose gel electrophoresis. The same amount of PCR products from each sample was pooled, end-repaired, A-tailed, and further ligated with Illumina adapters. The library was checked with Qubit and real-time PCR for quantification, as well as a bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on a paired-end Illumina platform to generate 250 bp paired-end raw reads. Paired-end reads were joined by using FLASH (Magoč and Salzberg, 2011). The DADA2 method (Callahan et al., 2016) was used for the noise reduction. ASVs (Amplicon Sequence Variants) were further filtered using QIIME2 software (Version 221 QIIME2–202202) and identified by using the Silva Database 138.1.

The 16S rRNA gene sequences are available at the Sequence Read

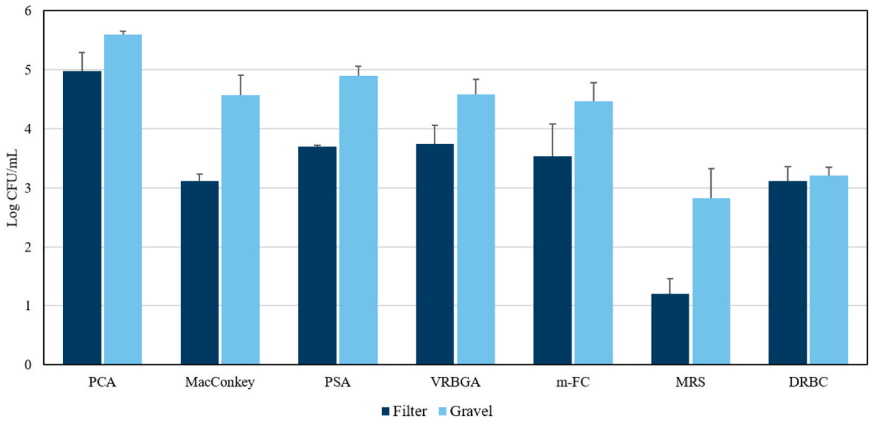

Fig. 2. Heterotrophic microflora (PCA), Gram-negative bacteria (MacConkey), Pseudomonadaceae (PSA), Enterobacteriaceae (VRBGA), Coli-aerogenes group (m-FC), lactic acid bacteria (MRS), yeast and moulds (DRBC) counts in the aquarium filter and gravel.

Archive (SRA) of the National Center for Biotechnology Information (NCBI), under accession number PRJNA1418103.

3. Results

3.1. Prey isolation and identification

Water collected from the aquarium was used for the prey isolation using MacConkey Agar. As the colonies shared the same appearance, a total of 5 colonies were randomly selected. By 16S rRNA sequencing, all isolates were *A. hydrophila* with at least a 99.74% similarity (Data not shown). *Aeromonas* species can be found in a variety of aquatic and environmental habitats, including sediment, estuaries, seaweed, sea grass, wastewater, drinking water, and food (Semwal et al., 2023). In fish, *A. hydrophila* causes diseases such as Motile Aeromonas Septicaemia (MAS), Haemorrhagic septicaemia, ulcer disease, or red-sore disease (Semwal et al., 2023; Gao et al., 2024).

3.2. BALOs isolation, identification, and characterization

Four predatory bacteria were isolated using *A. hydrophila* as prey. Conversely, no predation occurred against *E. coli* 32. Three strains out of four (ACV2, ACV3, and ACV4) exhibited an unusual behaviour. After a few stages of purification plating, these three strains no longer produced lytic plaques, despite being isolated from *A. hydrophila* enrichments. The strains were then successfully propagated using strain *E. coli* NCTC 10538. One plausible explanation is that autochthonous bacteria acting as prey might still be present in the aquarium water even after a 0.45 μm filtration. The circumstance that the strains produced lytic plaques but did not clarify the liquid enrichments further supports this theory. The four strains shared the same 16S rRNA sequence (data not shown). Therefore, only one 16S rRNA sequence (strain ACV1) was deposited in the GenBank database under the accession number PX498268.1. Alignment of the 16S rRNA gene sequences collected from GenBank database indicated that strain ACV1 was a member of the *Bdellovibrio*

Table 1
Results from the comparison of the 16S rRNA sequences of the selected isolates to the Bacterial 16S Ribosomal RNA Database (NCBI). Only the closest relatives obtained in each case are shown.

Sample	Medium	N. strains	Taxon	Closely related accession numbers Strain code in parentheses	Similarity (%)
Filter	wPCA	1	*Gottfriedia acidiceleris*	NR_043774.1 (CBD 119)	99.26%
			Gottfriedia solisivae	NR_159143.1 (NEAU-cbsb5)	99.26%
		1	*Bacillus wiedmannii*	NR_152692.1 (FSL W8–0169)	99.28%
			Bacillus proteolyticus	NR_157735.1 (MCCC 1A00365)	99.28%
			Bacillus sanguins	NR_175555.1 (BML-BC004)	99.28%
	MacConkey	5	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	99.44–99.80%
		1	*Pseudomonas lalkuanensis*	NR_179771.1 (PE08)	98.56%
		1	*Aquipseudomonas alcaligenes*	NR_114472.1 (ATCC 14909)	99.31%
	PSA	1	*Aquipseudomonas alcaligenes*	NR_114472.1 (ATCC 14909)	99.08%
		2	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	99.26–99.26%
	VRBGA	7	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	98.76–99.81%
	m-FC	9	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	99.07–99.73%
Gravel	wPCA	1	*Aminobacter anthyllidis*	NR_108530.1 (STM4645)	99.65%
		1	*Bacillus wiedmannii*	NR_152692.1 (FSL W8–0169)	99.18%
			Bacillus proteolyticus	NR_157735.1 (MCCC 1A00365)	99.18%
			Bacillus sanguins	NR_175555.1 (BML-BC004)	99.18%
		1	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	99.63%
		1	*Cytobacillus firmus*	NR_112635.1 (NBRC 15306)	97.55%
	MacConckey	5	*Pseudomonas lalkuanensis*	NR_179771.1 (PE08)	98.33–98.58%
		2	*Pseudomonas ullengensis*	NR_179010.1 (UL070)	98.32–98.76%
		1	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	99.63%
	PSA	2	*Pseudomonas lalkuanensis*	NR_179771.1 (PE08)	98.36–98.40%
		5	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	99.35–99.73%
		1	*Pseudomonas ullengensis*	NR_179010.1 (UL070)	97.93%
		2	*Aquipseudomonas alcaligenes*	NR_114472.1(ATCC 14909)	98.28–99.00%
	VRBGA	6	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	98.68–99.76%
	m-FC	2	*Pseudomonas lalkuanensis*	NR_179771.1 (PE08)	98.50–99.66%
		4	*Aeromonas hydrophila*	NR_074841.1 (ATCC 7966)	99.25–99.81%

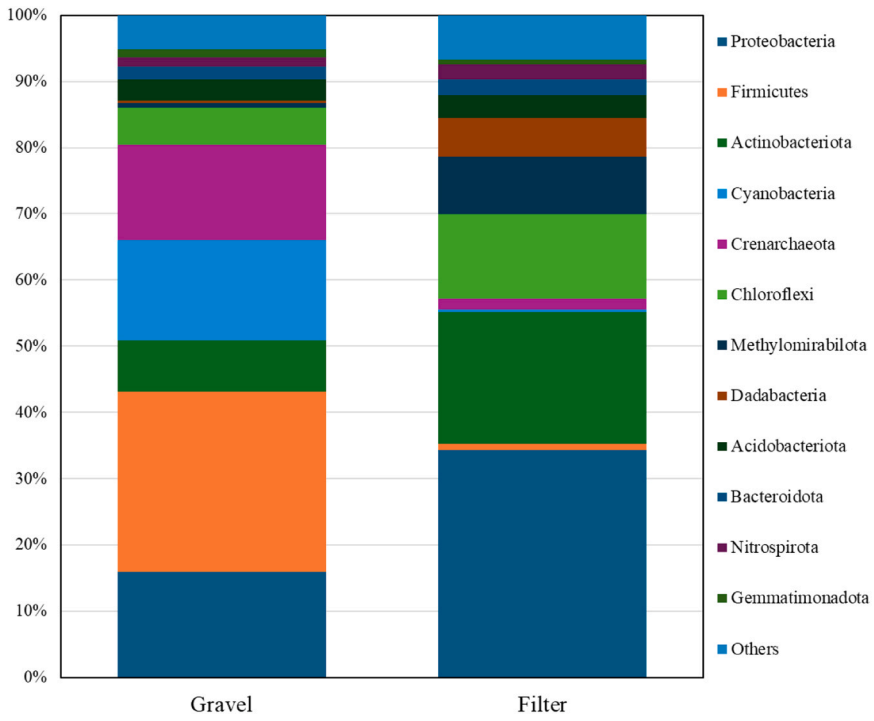

Fig. 3. Barplots showing the mean relative abundance of bacterial *phyla* in the aquarium filter and gravel. Only taxa with a mean relative abundance > 1% are plotted.

genus, showing 99.86% similarity to strain HCB337 (Accession CP178621.1) recently isolated from Chinese soil (Fig. 1).

Despite the different prey preferences, the four strains shared the same optimal temperature. Lytic plaques were never retrieved at 37°C, and at 20°C appeared only after 13 days. At 28°C, PFU/ml were in the range 2.54 ± 0.32 (ACV1) to 2.94 ± 0.16 Log PFU/ml (ACV3) after 5 days (Suppl. Fig. 1). Then, using primers M13 and M13-R2, the four strains were analysed using RAPD-PCR. Both primers were highly discriminating, allowing for obtaining quite complex patterns (Suppl. Fig. 2). In any event, RAPD profiles were very similar between them but highly divergent from the prey pattern.

3.3. Aquarium microbiome

Populations of heterotrophic bacteria in both filter and gravel were consistently higher than 10^5 CFU/ml. Except for yeast and moulds, count levels for the filter were constantly more than one Log higher than those reported for the gravel (Fig. 2). By culture-dependent methods, the microbiome appeared to be dominated by bacteria belonging to Pseudomonadaceae and Enterobacteriaceae, and specifically faecal coliforms, since differences between VRBGA and m-FC count were not significantly different (Data not shown). Counts on PCA were in line with population levels recorded on Tryptic Soy Agar by Sreenath et al. (2005) for the biofilter of a freshwater home aquarium hosting *Carassius auratus*. Colonies on both DRBC and MRS could be exclusively attributed to moulds.

By plates seeded with the highest dilutions, a total of 62 cultures were isolated and identified by 16S rRNA sequencing to analyse the dominant microflora in both the gravel and filter ecosystems. *A. hydrophila* was the most isolated species, regardless of the isolation medium. Members of this species accounted for 82 and 50% of the isolates in the filter and gravel, respectively (Table 1).

Pseudomonas ullengensis and *Pseudomonas lalkuanensis* were recently isolated from soil (Kim et al., 2021; Thorat et al., 2020). *Aquipseudomonas alcaligenes* (formerly *Pseudomonas alcaligenes*) is regularly reported as an opportunistic pathogen in fish populations under stressful conditions, such as overcrowding, low oxygen content, and high-temperature water (Bai et al., 2021).

Sequencing the V3–V4 hypervariable region of the bacterial 16S rRNA gene from aquarium gravel and filter samples generated 101,019 reads. In the gravel samples, eubacteria accounted for 85.42% of the total reads, while Archaea accounted for 14.57%. In the filter, eubacteria dominated, accounting for 98.25% of the total reads, while Archaea accounted for just 1.75% (Data not shown).

Firmicutes, recently renamed as Bacillota, dominated the gravel, followed by Proteobacteria and Cyanobacteria (Fig. 3). Bacillota, Proteobacteria, and Actinobacteriota (7.84% in the present study) have already been reported as dominant in freshwater filter systems and marine aquaria water (Raja et al., 2006; Sugita et al., 2005). Typically, Actinobacteria dominate freshwater environments (e.g., lakes, streams, etc.) (Embley and Stackebrandt, 1994), whereas Proteobacteria dominate ornamental fish aquarium waters (Smith et al., 2012).

The filter microbiota was rather different. Proteobacteria dominated (34.28%), followed by Actinobacteriota (19.93%), and Chloroflexi (12.68%). The phylum Chloroflexi has been associated with phenylethylamine degradation and denitrification in multiple tropical saltwater aquariums (Hu et al., 2022).

Archaea were essentially represented by Crenarchaeota, part of another branch of the domain now called Thaumarchaeota (Ren et al., 2019).

Firmicutes in the gravel were mainly represented by spore-forming bacteria (Fig. 4) belonging to Clostridiaceae (relative abundance 3.64%), Paenibacillaceae (3.52%), Bacillaceae (2.50%), and above all, Sporomusaceae (4.72%). The family Sporomusaceae has recently been retrieved from drinking water distribution pipes (Taligrot et al., 2025). Archaea were almost entirely represented by Nitrosopumilaceae (14.30%). This family has been reported at low levels in the external canister biofilter of a freshwater fish aquarium (Godzieba et al., 2024), but its potential role in the nitrogen cycle in closed aquatic ecosystems has not been highlighted before. Planktonic Nitrosopumilaceae and Poseidoniales have been found in a variety of marine habitats, from Antarctic seawater to temperate and subtropical waters of the North

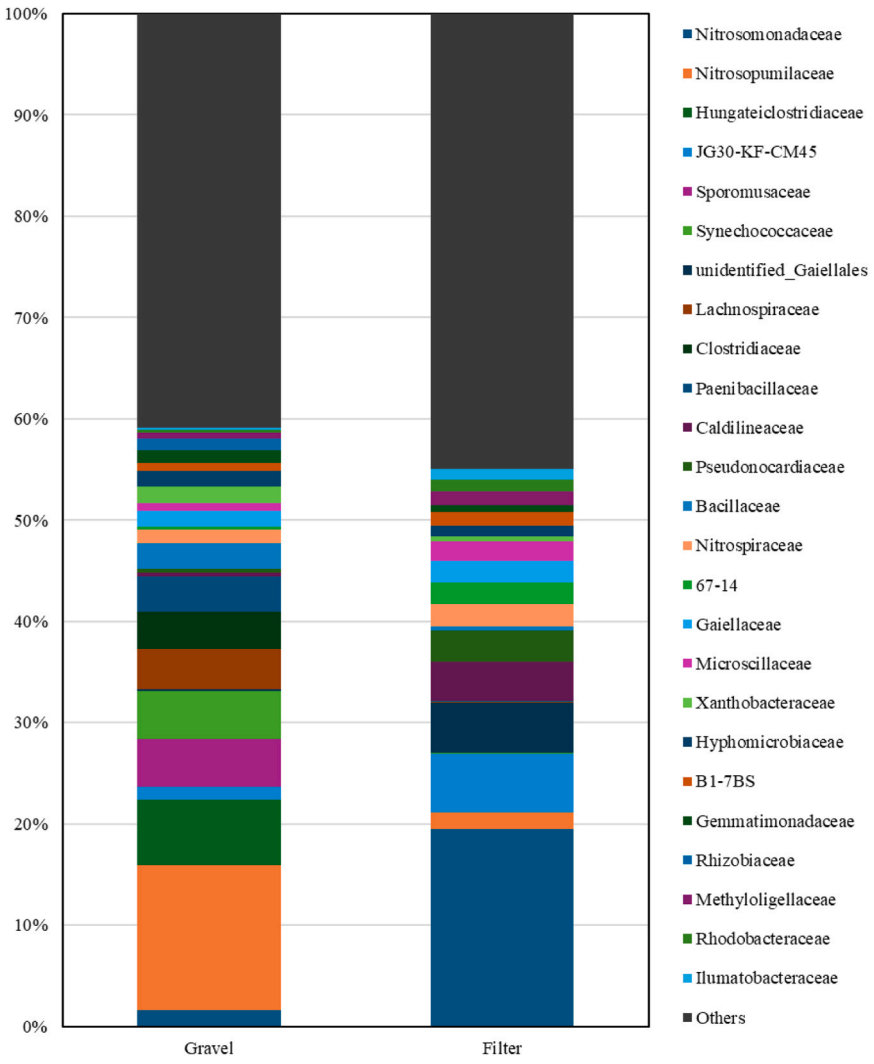

Fig. 4. Barplots showing the mean relative abundance of bacterial families in the aquarium filter and gravel. Only taxa with a mean relative abundance > 1% are plotted.

Pacific, and have been proven to be sponge symbionts (DeLong, 2021). In 2011, Sauder et al. (2011) sampled 27 standalone freshwater aquaria from homes and retail outlets using qPCR, revealing that Ammonia Oxidizing Archaea were the dominant population in most of the freshwater filters sampled and that this group could act alone in catalysing ammonia oxidation. Furthermore, according to Bagchi et al. (2014), Ammonia-Oxidizing Archaea exhibit a preferential growth on the fine sponge material of aquarium biofilters. In the present study, Archaea appear to be responsible for ammonium oxidation in the gravel but not in the filter, where the process is apparently carried out by Nitrosomonadaceae (relative abundance 19.52%). Anyhow, in a seawater aquarium, physicochemical parameters and microbial community dynamics have been proven to exert further influences on the relative abundances of nitrogen-transforming taxa (Bik et al., 2019). New evidence obtained by metagenome assembled genomes suggests that some lineages of marine Nitrosopumilaceae may be heterotrophic, instead of strict chemolithoautotrophs growing on ammonia (or urea) and CO_2 (Aylward and Santoro, 2020; Reji and Francis, 2020). Their presence in a simple system like an ornamental aquarium might surely help to settle this issue.

In the gravel sample, 3707 ASVs could be reported to Candidatus *Nitrosotenuis* (relative abundance 6.94%) (Suppl. Fig. 3). Sauder et al. (2018) provided the complete genome sequence of "Ca. *Nitrosotenuis aquarius*", an ammonia-oxidizing archaeon isolated from a freshwater aquarium biofilter. In the present survey, the obtained sequences did not match any of the several species associated with this newly proposed genus (Data not shown). In the filter, the highest relative abundance was recorded for Nitrosomonadaceae 966–1 (Suppl. Fig. 3), a typical ammonia-oxidizing bacterium recently isolated from a pilot-scale sludge alkaline anaerobic fermentation process enhanced by magnetite (Jiang et al., 2023).

4. Conclusions

Ornamental aquaria proved to be a complex microbial ecosystem hosting more than one uncommon and unexpected species. The dominance of *Aeromonas hydrophyla* in such an ecosystem has been highlighted since 2000 by Filler et al. (2000) and is unquestionably confirmed in the present study. Additionally, newly defined species such as *Pseudomonas lalkuanensis* and *Pseudomonas ullengensis* were also recovered. The isolation of potential new BALOs species paves the way to biotechnological applications. As these new strains prey on *A. hydrophyla* and *E. coli*, which are common fish pathogens, they may be profitably used as biocontrol agents in aquaculture.

CRediT authorship contribution statement

Giuseppe Blaiotta: Writing – original draft, Methodology. **Maria**

Aponte: Writing – review & editing, Conceptualization. **Ivan Ciliberti:** Methodology, Formal analysis, Conceptualization. **Raffaele Romano:** Writing – review & editing. **Lucia De Luca:** Formal analysis.

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Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2026.128594.

Data availability

No data was used for the research described in the article.

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