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Occurrence of *Vibrio mediterranei* Pujalte and Garay, 1986 in aquarium reared marine ornamental fish

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Short communication

Abstract

Vibrios are ubiquitous in the marine environment, some of which are opportunistic pathogens, while several others cause serious pathogenic conditions. They are among the bacterial pathogens that top the list of pathogens which lead to economic loss in aquaculture. Although the occurrence of bacterial diseases in marine aquaria is not well documented, disease conditions characteristic of bacterial pathogens were noticed in the captive fishes maintained in the marine aquarium functioning under Vizhinjam Research Centre of Central Marine Fisheries Research Institute (CMFRI). In view of the importance of supply of healthy organisms for commercial aquarium trade, attempts were made to isolate and identify bacterial pathogens from diseased and moribund fishes of the said aquarium. Three species of Vibrio such as *Vibrio furnissi, Vibrio fluvialis* and *Vibrio mediterranei* from the commercially important marine ornamental fishes–*Acanthurus* sp. (surgeon fish), *Amphiprion sebae* (sebae clown fish), *Heniochus acuminatus* (banner fish) and *Chaetodon auriga* (butterfly fish). Among these, *V. mediterranei* is a comparatively lesser demonstrated species. Biochemical characterization of these isolates was carried out for differentiation from other species of Vibrio. Previous reports of occurrence of the species are from corals and associated ecosystems. The usefulness of this species in marine culture systems as a potential probiont needs to be investigated.

Keywords: *Ornamental fish, Vibrio, bacterial isolate, coral, biochemical characterization, probiont*

Introduction

Culture and maintenance of marine ornamental fishes intensified consequent to their ever increasing demand in the international market. Domestic ornamental fish market in India is worth a billion dollars and the demand is on the rise (Kumar *et al.*, 2015). However, production failures in the culture systems often result from diseases due to various infectious agents, among which bacterial pathogens form a major component. Bacterial diseases due to Gramnegative organisms are the most common infectious problem of ornamental fishes (Lewbart, 2001). Of these, vibrios are particularly important as they are ubiquitous in the marine environment and are considered as opportunistic pathogens present as part of the normal microflora, occasionally capable of causing disease in wild and cultured fish (Hjeltnes and Roberts,

1993; Austin and Austin, 2012). Fish vibriosis is the general term given for diseases of fishes caused by bacteria belonging to *Vibrio* genus, outbreaks of which arise when a stressed fish is exposed to it. With rapid developments in mariculture operations, several species of *Vibrio* have been recognized as serious pathogens in economically important fish and shellfish. However, documentation related to communicable diseases in marine aquaria is scarce, except for a few reports on parasitic infestations, infections due to certain viral and fungal pathogens and some opportunistic bacterial pathogens (Giavenni *et al.*, 1980; Raja *et al.*, 2009; Lafferty *et al.*, 2015).

India's prospects in the marine ornamental fish trade depend to a very large extent on supply of wild caught fish for captive maintenance. The conservation challenges associated with this practice and the insufficient supply of hatchery produced seed can significantly affect the trade. In this context, studies were carried out on pathological conditions in marine ornamental fishes at the marine aquarium of the Vizhinjam Research Centre of Central Marine Fisheries Research Institute (CMFRI). Attempts were made to characterize the bacterial isolates from commercially important marine ornamental fishes- clown fish (*Amphiprion sebae*), surgeon fish (*Acanthurus* spp.), butterfly fishes (*Chaetodon* spp.), damsels (*Pomacentrus* spp.), parrot fishes and serranids.

Material and methods

Fishes were maintained in Fibreglass Reinforced Plastic (FRP) tanks of 1 to 5 t capacity, 1 t cement tanks and in rectangular glass aquaria of dimension 8 × 2 × 2 ft, all of which were provided with facilities for biological filtration and recirculation by airlift at the rate of 24 l/min. The stocking density depended on the size and behavior of fishes kept. The fishes were fed with boiled mussel meat twice a day.

A total of 544 diseased and dead fishes collected from the aquarium were examined for specific lesions associated with infections due to bacterial pathogens. They included commercially important finfish species belonging to families- Pomacentridae, Siganidae, Chaetodontidae, Callyodontidae, Serranidae, Acanthuridae and other miscellaneous groups. From these, moribund and recently dead fishes showing characteristic symptoms such as ulcerations, fin erosions and exophthalmic conditions were selected for microbiological investigations.

Samples were collected from internal organs such as kidney, liver and intestine and were inoculated in peptone broth (HiMedia), seawater agar media (composition of media: peptone 1%, agar-2%, ferric phosphate–a pinch, aged sea water, pH-7.2, 15 lbs, 20 minutes) and Thiosulphate Citrate Bile Salt (TCBS) agar (HiMedia), via broth culture and streak culture in agar plates. The broth cultures were allowed overnight incubation in a shaker followed by streaking in agar plates. The agar plate cultures also were incubated overnight at 37°C. The bacterial colonies obtained in the plates were further purified by the streak plate method. Following examination of individual morphological features of the colonies isolated from each infected fish, they were maintained in duplicate in agar slants for identification. Pure isolates maintained in agar slants were made free of contamination at frequent intervals by streak plating and sub culturing and were used for biochemical characterization. Basic genera level identification of bacteria was done following the scheme of Simidu and Aiso (1962) and Surendran and Gopakumar (1981). For characterizing the *Vibrio* group, the key of Alsina and Blanch (1994) was used.

Results and discussion

Bacterial isolates identified along with the source organisms from which they were obtained and the symptoms exhibited by the infected fish are given in Table 1. The percentage composition of the bacterial genera isolated from diseased marine ornamental fish is presented in Fig. 1. Vibrios including *Vibrio furnissi*, *Vibrio fluvialis* and *Vibrio mediterranei* constituted 18% of the total

Table 1. Bacterial isolates identified and the infected source fish

Isolate Code	Identified	Host fish	Symptoms
	Genus / species		
9-98B	*Alcaligenes*	*Acanthurus*	Ulcerations
10-98A	*Acinetobacter*	Serranid	Gill lesions
15-98A	*Flavobacterium*	*Apogon*	Fin erosions
15-98B	*Flavobacterium*	*Apogon*	Gill lesions
20 -98V	*V. furnissi*	*Acanthurus*	Ulcerations
20-98D	*Alcaligenes*	*Acanthurus*	Ulcerations
20-98W	*A. hydrophila*	*Acanthurus*	Ulcerations
20-98R	*S. marcescens*	*Acanthurus*	Ulcerations
23-98	*Alcaligenes*	Serranid	Ulcerations
29-98	*Pseudomonas*	*Acanthurus*	Haemorrhagic areas
40-98A	*V. fluvialis*	*Amphiprion*	Ulcerations
40-98B	*Pseudomonas*	*Amphiprion*	Ulcerations
40-98C	*A. hydrophila*	*Amphiprion*	Ulcerations
40-98D	*Pseudomonas*	*Amphiprion*	Ulcerations
42-98	*Alcaligenes*	*Acanthurus*	Ulcerations, Haemorrhages
71-98A	*Flavobacterium*	*Amphiprion*	Unilateral exophthalmia
71-98B	*Pseudomonas*	*Amphiprion*	Unilateral exophthalmia
78-98	*V. furnissi*	*Amphiprion*	Fin erosions
72-98	*Flavobacterium*	Damsel	Fin erosions
103-98	*Alcaligenes*	*Amphiprion*	Haemorrhagic areas
103-98A	Enterobacteriaceae	*Amphiprion*	Haemorrhagic areas
103-98B	*Acinetobacter*	*Amphiprion*	Haemorrhagic areas
115-98	*Pseudomonas*	Damsel	Deep ulcerations
1-99	*Acinetobacter*	*Acanthurus*	Fin erosions, Ulcerations
4-99	*Acinetobacter*	*Apogon*	Fin erosions
41-99	*Pseudomonas*	Serranid	Ulcerations, fin erosions
50-99A	*Flavobacterium*	Banner fish	Haemorrhages, fin erosions

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50-99B	*V. mediterranei*	Banner fish	Haemorrhages, fin erosions
61-99	*Pseudomonas*	*Amphiprion*	Fin erosions, ulcerations
61-99A	*V. mediterranei*	*Amphiprion*	Fin erosions, ulcerations
61-99B	*V. mediterranei*	*Amphiprion*	Fin erosions, ulcerations
68-99A	Enterobacteriaceae	*Chaetodon*	Ulcerations
68-99B	*Flavobacterium*	*Chaetodon*	Ulcerations
73-99	*V. mediterranei*	*Chaetodon*	Ulcerations
117-99	*Flavobacterium*	Squirrel fish	Ulcerations
124-99	*V. furnissi*	*Acanthurus*	Deep ulcerations
I51-99A	*Acinetobacter*	*Chaetodon*	Fin erosions
151-99B	*S. marcescens*	*Chaetodon*	Fin erosions, ulcerations
I51-99C	*Alcaligenes*	*Chaetodon*	Fin erosions
CFY	*Flavobacterium*	*Amphiprion*	Ulcerations, fin erosions
CY	*Flavobacterium*	*Amphiprion*	Ulcerations, fin erosions
LJ	*Flavobacterium*	*Apogon*	Ulcerations
OE	*Alcaligenes*	*Apogon*	Ulcerations
8-2K	*Flavobacterium*	*Amphiprion*	Fin erosions
9-2K	*Flavobacterium*	*Amphiprion*	Fin erosions

number of isolates. One among these, *Vibrio mediterranei* is of significance owing to the scarcity of reports on isolation of the same from marine aquaria or from aquacultured organisms in earlier literature. The biochemical tests used for identifying *V. mediterranei* are detailed in Table 2.

Previous reports on occurrence of *V. mediterranei* are from corals and abalones. They are considered as coral pathogens that play a pivotal role in coral bleaching (Thompson *et al.*, 2004). The ornamental fish maintained at the marine aquarium are generally collected from the southwest coast of India where the rocky and coralline substrates form ideal habitats for ornamental fishes. Buller (2014) has suggested that *V. mediterranei* is often associated with abalone and corals, such as the Mediterranean coral, *Oculina patagonica*. The association of the species with corals has been reported by Rubio-Portillo *et al.* (2014) as well.

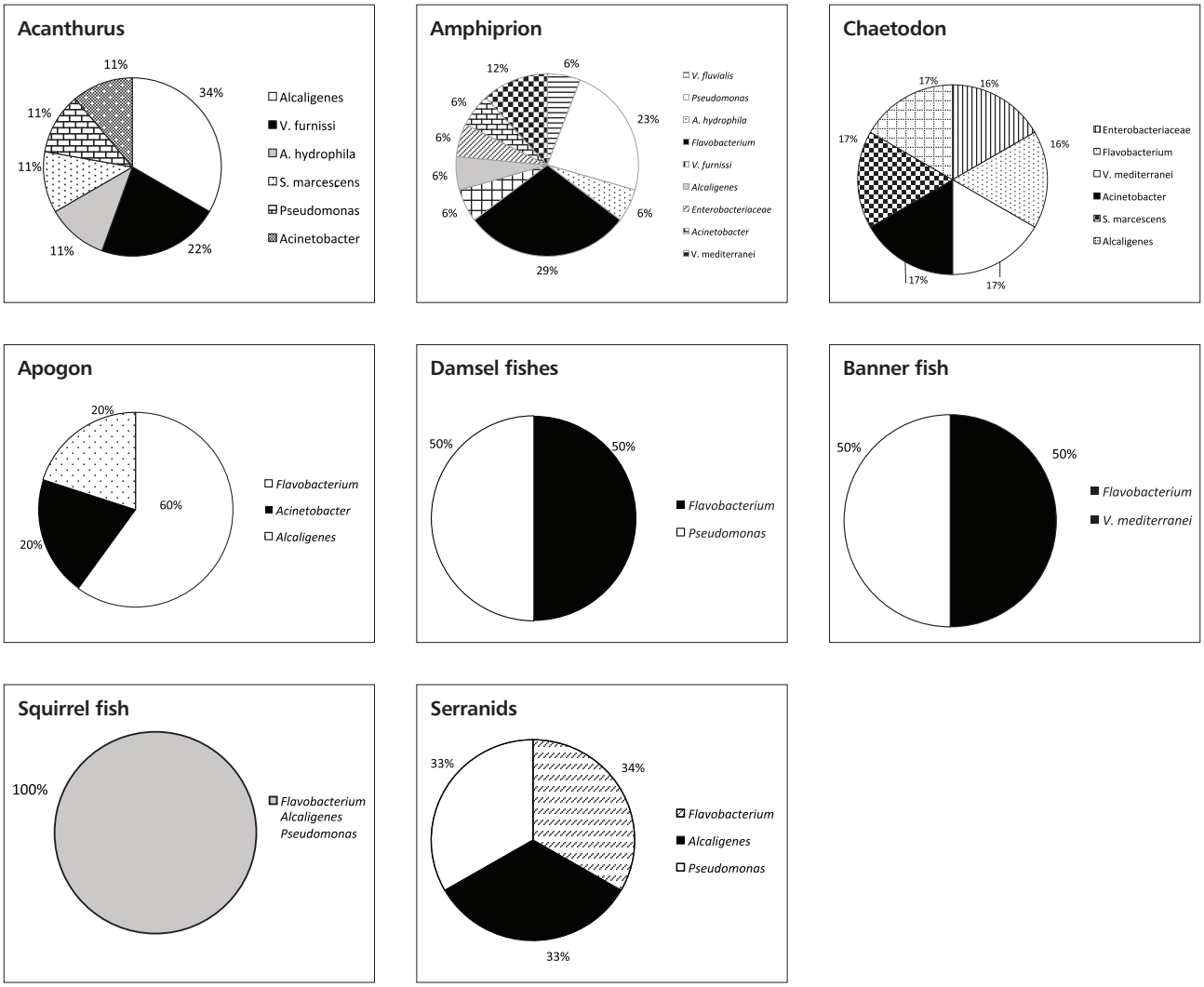

Fig. 1. Percentage composition of bacterial genera isolated from marine ornamental fishes

Table 2. Biochemical characteristics of *V. mediterranei* isolates

TEST	RESULTS			
Isolate code	50-99B	61-99A	61-99B	73-99
Penicillin (2.5 IU per disc)	R	R	R	R
H&L Fermentation	F	F	F	F
Oxidase	+	+	+	+
Hydrogen sulphide production	+	+	+	+
Indole	+	+	+	+
Starch hydrolysis	V	+	+	+
Gelatin liquification	-	-	-	-
Glucose	-	-	A	-
Sucrose	A	A	A	A
Lactose	-	-	-	-
Sorbitol	A	-	A	A
Arabinose	-	-	-	-
Urease	+	+	+	+
Growth at 5ºC	V	V	+	+
Growth at 37ºC	+	+	+	+
Growth at 0% NaCl	-	-	-	-
Growth at 5% NaCl	+	+	+	+
Growth at 7% NaCl	+	+	+	+
Citrate utilization	-	-	-	-
Arginine	-	-	-	+
Lysine	-	-	-	-
Ornithine	-	-	-	-
Methyl Red	+	-	+	+
Voges-Proskaur	-	-	-	-
0/129 Sensitivity	+	+	+	+
ONPG	-	+	-	-

R - Resistant
\+ - Positive
\- - Negative
V - Variable
F - Fermentation
A - Acid production

The presence of *V. mediterranei* was recorded from fishes caught off Chennai coast by Rajapandiyan *et al.* (2009). *V. mediterranei* is also represented as one of the halophilic vibrios isolated from healthy and brown ring disease affected manila clams (*Ruditapes philippinarum*) in the investigations by Castro *et al.* (2002). Gomez-Gil *et al.* (2004) reported this species to be implicated principally with disease outbreaks in shrimp larviculture facilities. Arunagiri *et al.* (2013) reported this species from seafood resources and from seawater, whereas Ravikumar and Meignanalakshmi (2013) recorded this species from seawater, sediment and plankton samples collected off Chennai coast. The above results demonstrate the significance of *V. mediterranei* as a component of bacterial flora present in seawater.

In the present study, *Vibrio* species were found to constitute 18% of the isolates, 50% of which were obtained from the clown fish, *A. sebae*. The widely used practical set of biochemical keys developed by Alsina and Blanch (1994) were useful for identifying the vibrios upto species level. However, revised and improved keys as well as availability of molecular methods could be explored further to define the diversity of vibrios in the marine environment. In the present study, *V. mediterranei* species was found cytochrome oxidase positive and in general showed fermentation in the Hugh and Leifson's medium without the production of gas. Hydrogen sulphide production was exhibited. Citrate utilization was negative for the isolates and they showed sensitivity to the vibriostatic compound, O/129. Cerda-Cuéllar *et al.* (1997) have reported that *V. mediterranei* exhibited positive results to indole and ONPG reaction and negative response to gelatin and citrate, in their studies on genotypic and phenotypic characterization of a new *Vibrio* species from juvenile turbot. Similar test results were obtained in the present investigations.

The results of biochemical characterization of the isolates indicated that growth at different concentrations of sodium chloride was one key character that could be used for differentiating the members of *Vibrio* genus. Fluctuations in salinity levels are found to influence the diversity and distribution of bacterial flora. Higher salinity levels are favorable for halophilic bacteria such as *Vibrio*, as demonstrated by Straub and Dixon (1993), in the case of bacterial flora of brine shrimp, *Artemia franciscana*. In the present study, the growth of these coral associated bacteria in higher levels of salinity, (5 and 7%) points to the ambient salinity in the natural environment.

Studies on the taxonomy of the species conducted earlier have shown that *Vibrio shiloi*, bacteria often associated with coral bleaching are closely related to *V. mediterranei*. The genotypic and phenotypic data presented in the study by Thompson *et al.* (2001) collectively suggested that *V. shiloi* Kushmaro *et al.*, 2001 should be considered as a later synonym of *V. mediterranei* Pujalte and Garay 1986. However, confirmation on the taxonomic status of the present isolate, by methods of molecular biology and comparisons with type cultures were not carried out.

The function of this species as a potential probiont has been investigated by some authors. In a study on the aerobic bacterial flora in the gut of turbot larvae and their influence on larval survival, by Huys *et al.* (2001), *V. mediterranei* Q40 strain had a distinct positive and reproducible effect on larval survival. It also seemed to play a role as the first colonizer of the gut of turbot larvae and prevented colonization of the gut by opportunistic bacteria. The role of this species in the inhibition of potential pathogens such as *Vibrio parahaemolyticus* is also documented (Carraturo *et al*., 2006). More studies on these aspects need to be conducted so as to explore the possible role of the species in biological control of various bacterial pathogens in aquaculture. Such efforts are worthy of consideration with regard to the

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maintenance of high value marine ornamental fish, which are extremely susceptible to bacterial infections.

The foregoing account illustrates the prevalence of *V. mediterranei* in the marine environment, while emphasizing the significance of newer techniques relevant for taxonomy of fish bacterial pathogens. Observations on the presence of these halophilic bacteria known for causing coral bleaching, in diseased marine aquarium fish, point at its possible role in causing diseases in other reef-associated fauna. However, more investigations are needed to confirm the pathogenicity of the species and to ascertain its role as a potential pathogen. Similarly, the inhibitory activity and probiotic potential of this bacterial species also need to be studied in detail.

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