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REVIEW

A review of the taxonomy and seroepizootiology of *Vibrio anguillarum*, with special reference to aquaculture in the northwest of Spain

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ABSTRACT: A review of the literature shows that although the number of serotypes of *Vibrio anguillarum* reported from different countries varies, most of the vibriosis outbreaks throughout the world are caused by only 2 serotypes: O1 and O2 (European serotype designation). The remaining serotypes, associated mainly with environmental samples such as water, sediment, phyto- and zooplankton, are generally non-pathogenic to fish. This raises the question as to whether the 2 major pathogenic serotypes of *V. anguillarum* (isolated only from diseased or carrier fish) should be considered opportunistic or obligate fish pathogens. In addition, information useful in epizootiological and vaccination studies is presented about the antigenic heterogeneity detected within serotype O2. In this review, emphasis is placed on the presence in the marine environment of vibrios (presumptively assigned to the *V. splendidus* and *V. pelagius*) species that are taxonomically and serologically related with *V. anguillarum* and some of which have been associated with disease in fish cultured on the Atlantic coast of Spain and Norway. The precise phylogenetic position of these *V. anguillarum*-like organisms are well as their actual threat to marine aquaculture remains to be clarified.

INTRODUCTION

Vibriosis is a serious disease problem in the fish culture industry in saline waters with a worldwide occurrence. In addition, it affects a great variety of species regardless of fish size, season, or source of seedlings.

Galicia (northwestern Spain) is a very important mariculture site. However vibriosis, caused by *Vibrio anguillarum* and *V. tubiashii*, is one of the most threatening bacterial infections limiting the production of marine fish and shellfish, respectively, in this area (Devesa et al. 1985, Bolinches et al. 1986, Lodeiros et al. 1987, Toranzo et al. 1987a).

The taxonomy and serology of the genus *Vibrio* have been the subject of continuous revision in recent years. Despite this, a large number of isolates are still referred to as *Vibrio* spp. In fact, there are vibrios in the estuarine environment taxonomically and serologically related to *V. anguillarum* and *V. tubiashii* (Bryant et al. 1986, Fouz et al. 1990), some of which are implicated in disease problems in marine fishes (Lupiani et al. 1989a). These vibrios are phylogenetically closely related and can be designated as *V. anguillarum*-like organisms (Larsen 1982, 1983, Bryant et al. 1986, Fouz et al. 1990).

Vibrio anguillarum and other related pathogenic vibrios have been considered common members of the estuarine and marine habitats and, hence, facultative fish pathogens. However, although 10 serotypes are known to occur in this species, only 2 of them (O1 and O2, European serotype designation) (Sørensen & Larsen 1986) have been isolated from diseased fish, the remaining serotypes having been detected in environmental samples. Therefore, the precise role of the marine environment as a reservoir of vibriosis has not been clarified.

In this paper, we review the rapidly growing body information on the taxonomy, serology, and ecology of *Vibrio anguillarum*. Our hope is that the review will contribute to an international harmonization of the seroepizootiological information on the vibrios, thus facilitating the formulation of vaccines that contain the strains and serotypes representative of the needs of a given area.

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TAXONOMY

Although *Vibrio anguillarum* is the most studied aetiological agent of vibriosis, other members of the genus *Vibrio* have been implicated in epizootics of cultured and wild marine fish and shellfish. Table 1 lists the published reports on vibriosis outbreaks in fish, molluscs, and crustacea in which *V. anguillarum* has been phenotypically identified as the causative organism; also listed are the geographic areas involved and the species affected. Unfortunately, in some of these reports confirmation of the identification by serology was not provided.

Some of the *Vibrio* strains pathogenic to larvae of bivalve molluscs were compared with *Vibrio* species isolated from fish and the marine environment including *V. anguillarum, V. ordalii, V. nereis, V. fluvialis, V. diazotrophicus,* and *V. splendidus* (Hada et al. 1984). It was found that the strains from shellfish were distinct phenotypically and genotypically from the other *Vibrio* spp., being assigned to the new species *V. tubiashii. V. tubiashii* differed from *V. anguillarum* mainly in its ability to degrade xanthine and tyrosine, both traits being unusual for the *Vibrio* genus. These 2 biochemical properties were also detected in the *Vibrio* strains responsible for mortalities of *Ostrea edulis* larvae in our area (Bolinches et al. 1986, Lodeiros et al. 1987).

The genus *Vibrio* has received much attention in recent years, resulting in an increase in the number of the species recognized. Eight of these species have been described as pathogens of fish and shellfish: *V. anguillarum, V. ordalii* (Schiewe et al. 1981) (formerly *V. anguillarum* biotype II, Harrell et al. 1976), *V. tubiashii* (Hada et al. 1984), *V. damsela* (Love et al. 1981), *V. vulnificus* biotype II (Tison et al. 1982) (formerly *V anguillarum* type B and *V. anguillicida,* Muroga et al. 1976, Nishibuchi et al. 1980), *V. carchariae* (Grimes et al. 1984), *V. salmonicida* (Egidius et al. 1986), and *V. cholerae* non-O1 (Muroga et al. 1979, Yamanoi et al. 1980).

Vibrio anguillarum, V. tubiashii, V. ordalii, and *V. damsela* share a great number of phenotypic characteristics, but they can be distinguished from each other using phenotypic traits. One current problem is whether the bacterium *V ordalii* should be regarded as *V. anguillarum* biotype II or whether it should be recognized as a separate species based on a 60% DNA homology with *V. anguillarum* (Schiewe et al. 1981, Tajima et al. 1985). We think that the strong serological cross-reactions of *V ordalii* with the serotype O2 of *V. anguillarum* (Ezura et al. 1980, Tajima et al. 1985, Toranzo et al. 1987b) together with the results of DNA-DNA hybridization, support the conclusion that *V. ordalii* is merely a biotype of *V. anguillarum.*

We have investigated by numerical taxonomy (Unweighted Pair Group Method with Arithmetic averages, UPGMA; Sneath 1972) the relationship among *Vibrio anguillarum, V. tubiashii,* and the estuarine vibrios using a total of 341 strains isolated from diseased fish and shellfish as well as from water, sediment, phyto- and zooplankton (Fouz et al. 1990). Twelve defined phena of environmental vibrios were obtained, their similarity values with *V. anguillarum* and *V. tubiashii* ranged from 77 to 83%. Most of these vibrios corresponded to different biotypes of *V. splendidus* and *V. pelagius* (Fouz et al. 1990). Similar results were reported by Larsen (1982, 1983) and Bryant et al. (1986). All of these findings suggest that the environmental vibrios represent phenotypic variants of a major and unique group which should be named *V. anguillarum*-like or atypical *V. anguillarum.* The *V. anguillarum*-like organisms differ from *V. anguillarum* in one or more of the following phenotypic traits: arginine dihydrolase, fermentation of sucrose, arabinose, and mannitol, gelatin hydrolysis, and resistance to ampicillin.

It is noteworthy that members of this group of vibrios (*V. splendidus* – *V. pelagius*) have recently been isolated in pure or mixed cultures from dead marine fishes cultured in the Atlantic Coast of Spain and Norway (Toranzo, Fouz & Gravningen, unpublished data). In fact, we have demonstrated that a strain of *V. splendidus* was implicated in a new pathologic syndrome of bacterial and viral etiology that occurred in both juvenile and adult turbot in different hatcheries in our area (Lupiani et al. 1989a). Experimental infections confirmed the pathogenicity of this *V. splendidus* strain (Lupiani et al. 1989b) which differed from *V. anguillarum* in its inability to ferment sucrose and mannitol and to hydrolyse gelatin.

On the basis of rRNA phylogenic studies, McDowell & Colwell (1985) reported that the species *V. anguillarum, V. damsela,* and *V. pelagius* should be transferred into the newly proposed genus *Listonella.* However, further analysis of these data using a cluster program (Nearhos & Fuerst 1987) did not support this designation because simultaneous analysis of sequence data from related vibrios such as *V. ordalii* and *V. tubiashii* were not conducted. Had McDowell and Colwell included these species in their study, their conclusions on the phylogenetic relationships might have been different.

SEROLOGY

The importance of serology in epizootiological and ecological studies of *Vibrio anguillarum* was emphasized by Pacha & Kiehn (1969) who described 3 serotypes: Northwest salmonid vibrios (Serotype 1), European vibrios (Serotype 2), and Pacific herring vib-

Table 1. *Vibrio anguillarum*. Vibriosis outbreaks in cultured fish and shellfish of different countries

Species	Country	Source
FISHES		
Pacific salmon		
Oncorhynchus kisutch	USA	Rucker et al. (1953)
		Fryer et al. (1972)
		Harrell et al. (1976)
		Strout et al. (1978)
	Japan	Tajima et al. (1981, 1985)
	Spain	Toranzo et al. (1987a)
O. keta, O. nerka, O. gorbuscha	Canada	Evelyn (1971)
O. masou, O. rhodurus	Japan	Tajima et al. (1985)
O. tshawytscha	USA	Cisar & Fryer (1969)
	Canada	Evelyn (1971)
Atlantic salmon		
Salmo salar	Norway	Håstein & Holt (1972)
Trout		
Oncorhynchus mykiss	USA	Ross et al. (1968)
(formerly *Salmo gairdneri*)	Japan	Muroga & Egusa (1973)
		Kitao et al. (1983)
		Tajima et al. (1985)
	Italy	Giorgetti & Ceschia (1982)
	Norway	Holt (1970)
		Egidius & Andersen (1977)
	Denmark	Larsen (1983)
		Larsen & Mellergaard (1984)
		Sørensen & Larsen (1986)
	Spain	Toranzo et al. (1987a)
Salmo trutta	Scotland	Smith (1961)
		Håstein & Smith (1977)
Turbot		
Scophthalmus maximus	Scotland	Horne e al. (1977)
	Spain	Devesa et al. (1985)
		Toranzo et al. (1987a)
Striped bass		
Morone saxatilis	USA	Toranzo et al. (1983)
Winter flounder		
Pseudopleuronectes americanus	USA	Levin et al. (1972)
Cod		
Gadus morhua	Norway	Egidius & Andersen (1984)
	Denmark	Larsen (1983)
		Larsen & Jensen (1979)
		Larsen & Mellergaard (1984)
		Sørensen & Larsen (1986)
Red sea-bream		
Pagrus major	Japan	Muroga & Tatani (1982)
European eel		
Anguilla anguilla	Norway	Rodsaether et al. (1977)
Japanese eel		
Anguilla japonica	Japan	Kitao et al. (1983)
		Tajima et al. (1985)
Saithe		
Pollachius virens	Norway	Egidius & Andersen (1978)
Gilthead sea-bream		
Sparus aurata	Israel	Paperna et al. (1977)
Sea mullet		
Mugil cephalus	Scotland	Rodgers & Burke (1981)

Table 1 (continued)

Species	Country	Source
FISHES		
Seriola		
Seriola quinqueradiata	Japan	Jo et al. (1979) Tajima et al. (1985)
Channel catfish		
Ictalurus punctatus	USA	Lewis (1985)
Milkfish		
Chanos chanos	Taiwan	Huang (1977) Chen et al. (1985)
Ayu		
Plecoglossus altivelis	Japan	Muroga & Egusa (1967, 1970) Mifuchi et al. (1983) Tajima et al. (1985)
	Taiwan	Kuo et al. (1976)
Tilapia		
Oreochromis aureus	Kuwait	Tareen (1984)
MOLLUSCS[a]		
European oyster		
Ostrea edulis	USA Great Britain Spain	DiSalvo et al. (1978) Jeffries (1982) Bolinches et al. (1986) Lodeiros et al. (1987)
Japanese oyster		
Crassostrea gigas	USA Australia Great Britain	DiSalvo et al. (1978) Garland et al. (1983) Jeffries (1982)
American oyster		
Crassostrea virginica	USA	Tubiash et al. (1965, 1970) Elston (1979) Elston et al. (1981) Brown & Losee (1978) Brown (1981)
Clam		
Mercenaria mercenaria	USA	Tubiash et al. (1965, 1970)
CRUSTACEA		
Lobster		
Homarus americanus	USA	Bowser et al. (1981)
Shrimp		
Penaeus sp.	USA	Lewis (1979)

[a] Some of the isolates from molluscs, previously identified as *Vibrio anguillarum*, have been assigned to the species *V. tubiashii* (Hada et al. 1984)

rios (Serotype 3). Since then, several serotyping systems based on the slide agglutination method with thermostable O antigens have been developed. Up to 1986, 3 serotypes were recognized in Norway (Johnsen 1977), 2 or 3 in the United States (Harrell et al. 1976, Strout et al. 1978), and 9 in Japan (Kitao et al. 1984). Sørensen & Larsen (1986) recommended an international harmonization of the diagnostic efforts and presented a new antigenic typing scheme in which 10 distinct O serotypes of *V. anguillarum* were recognized. However, most of the isolates associated with diseased fish throughout the world belonged to the serotypes O1 and O2 (see references in Table 2). As shown in Table 2, these 2 major pathogenic serotypes have been given different names by different authors, making the coordination of epizootiological studies and vaccination programs in the different countries difficult. Actually, with the exception of laboratories in Japan and Taiwan, most of the investigators follow the scheme of Sørensen & Larsen. This scheme is in agree-

Table 2. *Vibrio anguillarum*. Serotyping systems based on the thermostable O antigens. Designations of the major pathogenic serotypes used by various authors

Country	Serotype designation		Source
Europe	Group 1	Group 2	Johnsen (1977)
	O2	O1	Sørensen & Larsen (1986)
USA	European vibrios	Northwest salmonid vibrios	Pacha & Kiehn (1969)
	Type II	Type I	Harrell et al. (1976)
	569 Group	775A Group	Strout et al. (1978)
Japan	I	III	Kusuda et al. (1975, 1981)
	A	C	Aoki & Kitao (1978) Kitao et al. (1983, 1984)
	J-O-1	J-O-3	Ezura et al (1980) Tajima et al. (1985)
Taiwan	T-O-V	T-O-I	Chang & Kou (1983) Song et al. (1988)

ment with the American serotyping system but it uses Arabic rather than Roman numerals and it employs the prefix 'O' to avoid confusion with capsular or flagellar antigens.

Our studies on the serological relationship within *Vibrio anguillarum* strains indicated that the isolates from O1 serotype constitute a homogenous group by the Double Immunodiffusion (IDD), Dot Blot Assay (DBA), and Enzyme Linked Immunoabsorbent Assay (ELISA) methods (Bolinches et al. 1990). However, within serotype O2, 2 different patterns of serological reactions were detected based on O antigens. These 2 antigenic entities were designated as the O2 α and O2 β subgroups. More recently, we have extended this research using the DBA on a large number of isolates from different sources and geographic areas (Fouz et al. 1989). It was found that the majority of *V. anguillarum* O2 strains (75%) belonged to the O2α subgroup and that this subgroup occurred in both salmonid and non salmonid fishes. In contrast, the O2β strains occurred mainly in non salmonid fishes (turbot, saithe, cod, and striped bass). In addition, we demonstrated that these 2 subgroups (O2α and O2β) were the same as subgroups O2a and O2b detected by Danish investigators using immunoelectrophoretic methods (Rasmussen 1987). The serological heterogeneity evident in strains of serotype O2 was greater than that found in serotype O1 and was consistent with their greater variability in phenotypic characteristics (Fouz et al. 1989).

Since 1983, several epizootics of vibriosis affecting turbot, coho salmon and rainbow trout have occurred in different locations on the Galician Coast (Table 3). Studies on the *Vibrio anguillarum* serotypes involved indicated that until 1987, regardless of the species of fish and culture site, practically all of the *V. anguillarum* strains isolated belonged to serotype O1. However, from 1988 onwards isolations of the *V. anguillarum* serotype O2α were made from turbot. This serotype has been reported as the predominant type in marine fishes in Denmark (Sørensen & Larsen 1986) and Norway (Wiik et al. 1989, Toranzo, Barja, Fouz & Gravningen, unpubl.) and its occurrence on the Galician coast may be a consequence of importations of fingerlings from nordic countries.

As shown in Table 3, all serotype O1 strains were arabinose positive, and harbored the 47 megadalton virulence plasmid similar to the pJM1 plasmid reported in *Vibrio anguillarum* 775 (Crosa 1980, Tolmasky et al. 1985, Toranzo et al. 1987a). In contrast, the O2 strains failed to ferment arabinose and lacked high molecular weight plasmids, indicating a chromosome-mediated virulence mechanism (Lemos et al. 1988). Similar findings were reported by Wiik et al. (1989) for the O1 and O2 *V. anguillarum* strains isolated in Norway. However, the existence of some *V. anguillarum* O1 strains lacking virulence plasmid (Lemos et al. 1988, Wiik et al. 1989) indicates that the presence of pJM1-like plasmids cannot be used as an indicator of serology.

Interestingly, our data on the serological relationships between the pathogenic serotypes of *Vibrio anguillarum* and the environmental vibrios (Fouz et al. 1990) indicated that all of the antisera raised against strains from each of the *V. anguillarum*-like phena displayed cross-reactions with *V. anguillarum* serotype O2 but not with serotype O1.

EPIZOOTIOLOGICAL STUDIES

The reservoir of the pathogenic serotypes of *Vibrio anguillarum* in the aquatic environment is uncertain because few data on the ecology and distribution of organism exist.

Table 3. *Vibrio anguillarum*. Epizootics in 8 different seawater ongrowing facilities (designated as A to H) located on the Northwest Atlantic Coast of Spain. Occurrence of pathogenic serotypes. Positive reaction (+) and presence of the virulence plasmid; NT: not tested

Strain	Host	Ongrowing farm	Year	Serotype	Arabinose fermentation	Virulence plasmid
R-82	Turbot	A	1983	O1	+	+
RV-22	Turbot	B	1985	O2β[a]	–	–
RT-32	Turbot	A	1985	O1	+	+
SO-86.3	Pacific salmon	C	1986	O1	+	+
SO-86.9	Pacific salmon	C	1986	O1	+	+
TM-14	Rainbow trout	C	1986	O1	+	+
TM-52	Rainbow trout	C	1986	O1	+	+
RP-13	Turbot	D	1987	O1	+	+
RP-51	Turbot	D	1987	O1	+	+
RP-63	Turbot	D	1987	O1	+	+
RM-21	Turbot	E	1987	O1	+	+
RP-81	Turbot	D	1987	O1	+	+
RC-52	Turbot	F	1988	O1	+	+
RC-71	Turbot	F	1988	O2α	+	–
RG-111	Turbot	G	1988	O2α	–	–
RG-121	Turbot	G	1988	O2α	–	–
RC-91	Turbot	F	1988	O1	+	+
RO-32	Turbot	C	1988	O1	+	NT
RG-133	Turbot	G	1988	O2α	–	–
RG-141	Turbot	G	1988	O1	+	+
RG-161	Turbot	G	1988	O1	+	+
RG-181	Turbot	G	1989	O2α	–	–
RH-81	Turbot	E	1990	O1	+	+
TH-10.1	Rainbow trout	C	1990	O1	+	+
RPH-23.1	Turbot	H	1990	O2α	–	–
RC-16.1	Turbot	F	1990	O1	+	NT

[a] The only strain isolated in an experimental culture facility

Muroga et al. (1984) conducted a 4 yr study on the incidence of *Vibrio anguillarum* in wild ayu fingerlings and found that fish caught in seawater or brackish water harbored the microorganism at significantly higher rates than fingerlings caught a in freshwater lake. From a total of 9574 fish examined, *V. anguillarum* was detected in only 168 (1.7%). In addition, infection tests revealed that only the strains belonging to the major pathogenic serotypes J-O-1 and J-O-3 (i.e. O2 and O1, respectively), were virulent.

Muroga et al. (1986) investigated the prevalence of *Vibrio anguillarum* in the environment and observed that this organism was often detected from seawaters of the Inland Sea of Japan but not from the freshwater of ayu ponds. This held true even during vibriosis outbreaks. Interestingly, the majority of the strains were non-pathogenic and non-typable as to O-serotype.

With ayu, the usual source of the infection in freshwater was the fingerlings captured from marine or brackish water (Muroga et al. 1984, 1986). However on some occasions it was shown that the marine rotifer (*Brachionus plicatilis*), fed to ayu larvae, was the source of the pathogen in the freshwater ponds (Tabata et al. 1982). Similarly, it seems probable that vibriosis outbreaks in trout cultured in freshwater in Italy (Giorgetti & Ceshia 1982) were due to the use of wet marine fish as food.

Sørensen & Larsen (1986), in a 5 yr study, examined more than 500 *Vibrio anguillarum* strains from feral and farmed fish, as well as from seawater, sediment, and invertebrates. They found that whereas the serotypes O1 and O2 were most commonly isolated from diseased fish (in cultured salmonid fish and feral marine fish, respectively), the environmental strains belonged to the remaining 8 serotypes or were nontypable. Similar findings were observed in another seroepizootiological survey conducted by Larsen et al. (1988) in rainbow trout at a Danish mariculture facility and in feral fish caught close to the facility. Similarly, Tajima et al. (1988) studied the distribution of *V. anguillarum* in coho salmon and their culture environment and detected 58 *V. anguillarum* from a total of 5337 *Vibrio* isolates obtained. Only the strains originating from pen-reared fish corresponded to the pathogenic serotype J-O-3 (i.e. serotype O1) and the isolates from seawater, mud,

and plankton corresponded to serotype J-O-8, a low-virulence serotype.

It is noteworthy that in the majority of these epizootiological studies, the pathogenic serotypes of *Vibrio anguillarum* could not be detected from carrier fish or environmental samples without an initial concentration or enrichment of the samples. This probably explains why, using direct isolation on agar plates, we were unable to recover serotypes O1 and O2 from the marine environment (Fouz et al. 1990).

Other epizootiological investigations focused on the factors affecting the stability of *Vibrio anguillarum* in the aquatic environment. Muroga & Egusa (1967), Evelyn (1971), and Itami & Kusuda (1984) evaluated the viability of *V. anguillarum* in different NaCl concentrations and found that optimum growth occurred in 1 to 2% salt. Larsen (1984) studied the interaction of environmental parameters such as temperature, pH, salinity, and incubation time on the growth of different isolates of *V. anguillarum*. Although their results varied according to the origin of the strains examinated, in general the optimal values were pH 7, 25°C, and 2% NaCl. Our survival studies in estuarine water (Toranzo et al. 1982) indicated that *V. anguillarum* had the ability to multiply within the first 4 d of the experiment. After this, there was a gradual decline in cell numbers, viable cells being detectable after more than 100 d. Muroga et al. (1986) found that the microorganism persisted in seawater for more than 15 d without a decrease in viable counts; however, it perished within 3 to 5 h in freshwater.

It should be emphasized that in these environmental studies an adaptive property among isolates of *Vibrio anguillarum* to distinct NaCl concentrations and temperatures was observed. Therefore, in agreement with Larsen (1984), we consider that *V. anguillarum* strains from various geographic areas have their own temperature and salinity optima and they occur in their niches at a characteristic density (viable cells ml^{-1}), usually showing a moderate yearly variation.

Another important ecological parameter determining the growth of *Vibrio anguillarum* is the load of organic matter in the water. Organic matter provides nutrients and solid surfaces for attachment and colonization (first step in the development of an infection). In order to evaluate the impact of organic pollution on the prevalence of *V. anguillarum* in the aquatic environment, Danish investigators (Larsen 1982, Larsen & Willeberg 1984) monitored over a 3 yr period the annual cycle of this microorganism in waters and sediments of coastal areas with different degrees of pollution. In general, the number of presumptive *V. anguillarum* in polluted areas was 10 to 100 per ml of water and 10^3 to 10^4 per g of sediment whereas the corresponding values for the unpolluted sites were 1 to 10 per ml water and 10^2 to 10^3 per g sediment. According to these results, fish living in polluted areas are exposed to a 10 times higher risk of vibriosis. The data suggest that the *V. anguillarum* content of the water might be used to indicate the level of organic pollution and the risk of infectious disease in wild and cultured fish. Larsen & Willeberg (1984) recommended that 10^4 *V. anguillarum* per 100 ml should be the upper acceptable level for selecting mariculture sites. However, we should point out that based on the tests used by these authors for the identification of the *Vibrio* isolates, *V. anguillarum*-like organisms probably contributed to the bacterial counts.

It has been suggested that studies on the ecology of *Vibrio anguillarum* should focus on virulence markers such as factors associated with host adhesion and colonization (Larsen et al. 1988). Hemagglutinating activity in addition to hydrophobic surface properties are sometimes associated with virulence. These authors reported that most of the O1 strains were non-hemagglutinating and that the majority of the O2 strains agglutinated a broad spectrum of erythrocytes. They also found that the O1 isolates were more hydrophobic than the O2 strains. These findings differ from our results (Santos et al. 1990) which showed that some *V. anguillarum* serotype O1 isolates from diseased fish displayed hemagglutinating activity and that the O2 isolates displayed more hydrophobic tendencies than serotype O1 strains. Because of these discrepancies (which may be attributed to the different origin of the isolates) we consider that these properties would be unreliable as virulence markers in seroepizootiological studies.

CONCLUSIONS

All the epizootiological data indicate that *Vibrio anguillarum* can be ubiquitous in the marine environment, but that only certain serotypes (e.g. O1 and O2) in the *V. anguillarum* population (which occur in a very low proportion in a particular reservoir) possess the genetic potential to successfully establish themselves in fish and cause disease (generally in fish with a lowered resistance). This raises the question whether all serotypes of *V. anguillarum* can be considered as opportunistic fish pathogens. In addition, the antigenic heterogeneity detected within serotype O2 emphasizes the importance of a harmonizing the terminology used in seroepizootiological studies so that vaccination programs in the different countries can be facilitated.

The existence in the marine environment of *Vibrio anguillarum*-like organisms, some of which are associated with fish disease, indicates the need for further investigations to determine: (1) the precise taxonomic position of these groups of vibrios, (2) their possible serological relationship with the environmental sero-

types of *V anguillarum*, and (3) their potential as pathogens for fish and shellfish.

The ecological studies on the stability of *Vibrio anguillarum* in different water types demonstrated that the seawater is an important vehicle of transmission of this microorganism and, hence, that epizootics of vibriosis can take place far away from the source of infection.

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