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A field report on vibrio disease of Seabass (*Dicentrarchus labrax*) in the South of France

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Abstract — *Vibrio anguillarum* was isolated from diseased seabass reared in aquatic farms (hatcheries and grow-out farms) located along the French Mediterranean coast. A representative strain (V62) differs from the French *V. anguillarum* strain (V408) by its ability to grow on a 0 ‰ salinity medium. The pathogenicity of the representative strain for sea bass was confirmed by injections and bath challenge. The V62 strain reacted with *V. anguillarum* antiserum (V408) and 84 % of the isolated strains from diseased sea bass agglutinated with anti V62 serum.

INTRODUCTION

Six French aquatic farms (3 hatcheries and 3 grow-out farms) located along the Mediterranean coast have been found to be faced with bacterial disease of seabass since 1986.

* In hatcheries (water temperature 20°C), all the rearings are affected with bacterial problems following the weaning period (0.2 to 0.5 g). A good antibiotherapy (Oxytetracycline 70 mg/kg/day for 8 to 10 days) is able to stop the disease. Nevertheless, mortalities still reach 15 to 20 % (from 0.2 to 2 g). Diseased animals show haemorrhagic symptoms (red mouth disease) and ulcerative lesions of the fins.

* In grow-out farms, mortalities usually occur in Spring or Autumn, after handling or sudden thermic variations. Estimated mortality reaches about 10 % (on an average general scale) during the first year of rearing, but only occurs in few cases during the second one. Diseased fishes show marked ulcerative lesions near the ventral zone or along the body side (Ulcer disease). A good antibiotherapy (Oxytetracycline, Furazolidone or Oxolinic acid) is also needed to stop the disease.

MATERIEL AND METHODS

Bacterial investigations

Bacterial samples

One hundred and thirty three bacterial samples, isolated from the blood, liver and skin of live diseased animals were analysed by standardized kits (API 20E, API 20NE, ADH-LDC-ODC kits from the Pasteur Institute). A presumptive bacterial identification (*Vibrionaceae*, *Pseudomonaceae* and *Enterobacteriaceae*) was done by using Bergey's manual procedure. For the *Vibrionaceae* the most accurate identification was made according to West and Colwell's scheme (1984) and to Reichenbach and Dworkin's (1981) for the Myxobacteria. Presumptive *Vibrio anguillarum* strains were then checked with the French vibrio 408 antiserum (Baudin-Laurencin 1981), and with the US V/408, V/775 antiserum.

Filamentous-like bacterias isolated from the skin did not show characteristics of mixobacterias when cultured on Anacker and Ordal medium (Anacker and Ordal 1959). These motile, oxydo-fermentative and O129 sensitive bacterias were further classified as *Vibrio* species.

Pathogenicity tests

Intraperitoneal inoculation

A saline (2.5 % NaCl) suspension containing 10^6 CFU/ml of the representative V62 strain was injected in the abdominal cavity (IP inoculation of $3 \cdot 10^5$ bacteria per fish) of 30 seabass weighing from 14 to 20 g. The water temperature was maintained at 20°C. A bacterial control is made on the kidney of moribond fishes.

Scarification, intradermal and bath challenge

Scarification : fishes weigh 15 g have been scarified on the body side. The lesion is then plugged with a sterile gauze (control group) or with a gauze soaked with salt water containing 10^8 bacterias/ml. The water temperature is maintained at 20°C (group 1) or raised from 19 to 25°C (group 2), or from 15 to 21°C (group 3 and control group).

Intradermal (ID) inoculation : 0.1 ml of a saline (2.5 % NaCl) suspension containing 10^6 bacteria/ml is injected to six fishes weighing 250 g (ID inoculation of 10^5 bacterias per fish). The control group is inoculated with a sterile saline (2.5 % NaCl) solution. The water temperature is 20°C.

Bath challenge : 30 seabass weighing 1 g have been bathed for 1 hour in salt water containing 107 bacterias/ml. The water temperature is 20 °C.

Epidemiology

Serological characterization of the *V. anguillarum* strain previously isolated (V62) was done by using an anti V62 strain rabbit serum supplied

by the Laboratoire National de Pathologie des Animaux Aquatiques (LNPA) de Brest (Baudin Laurencin pers. comm.). Seventy presumptive *V. anguillarum* strains showing the same biochemical character as the V/62 reported strain, have been tested by using the V/62.

Geographical repartition of the disease : various hatcheries and grow-out farms of seabass affected with vibriosis have been controlled by using the anti V62 serum in 1987.

RESULTS

Bacterial investigations

Strains of *Vibrio anguillarum* (representative strain V62) were isolated from 45.5% of the samples (fig. 1). The biochemical characters of the representatives strains (*V. anguillarum* V/62, *V. alginolyticus* and *V. parahaemolyticus*) are listed in table 1. The V62 strain reacts with the V408 antiserum as well as with the V775 one.

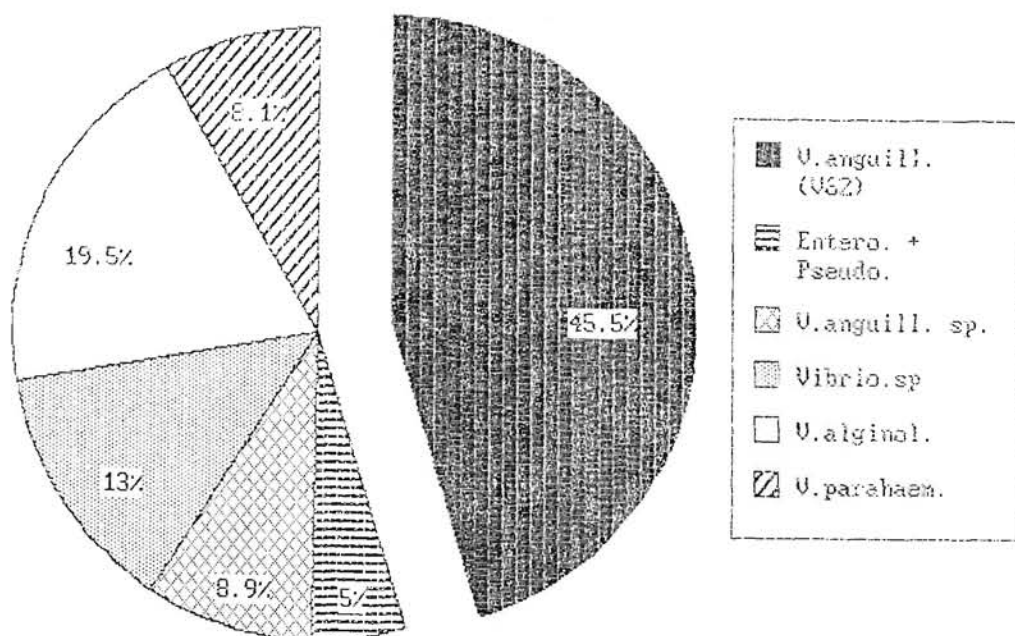


Figure 1. — Total bacteria distribution.

Bacterias identified as *V. fishery*, *V. vulnificus* or *V. proteolyticus* were classified in the *Vibrio* sp. group.

The percentage of the different bacterial species depends on which organs are sampled (fig. 2). *Vibrio anguillarum* is predominant in the blood and liver of infected animals and is present but not predominant in the skin, where *V. alginolyticus* is found in 36% of the samples.

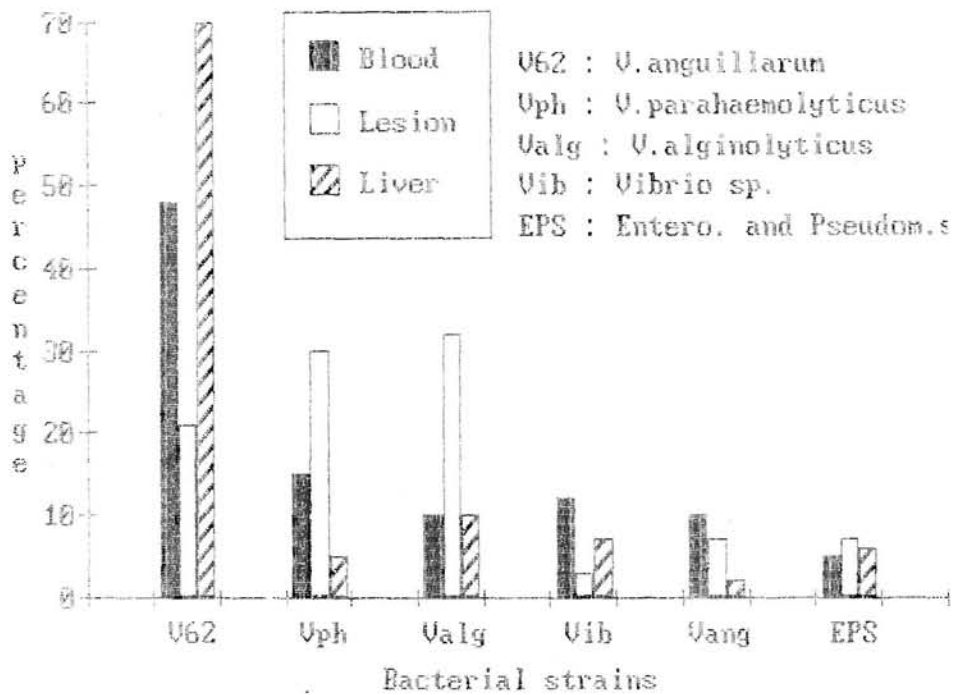


Figure 2. — Bacterial distribution in organ samples.

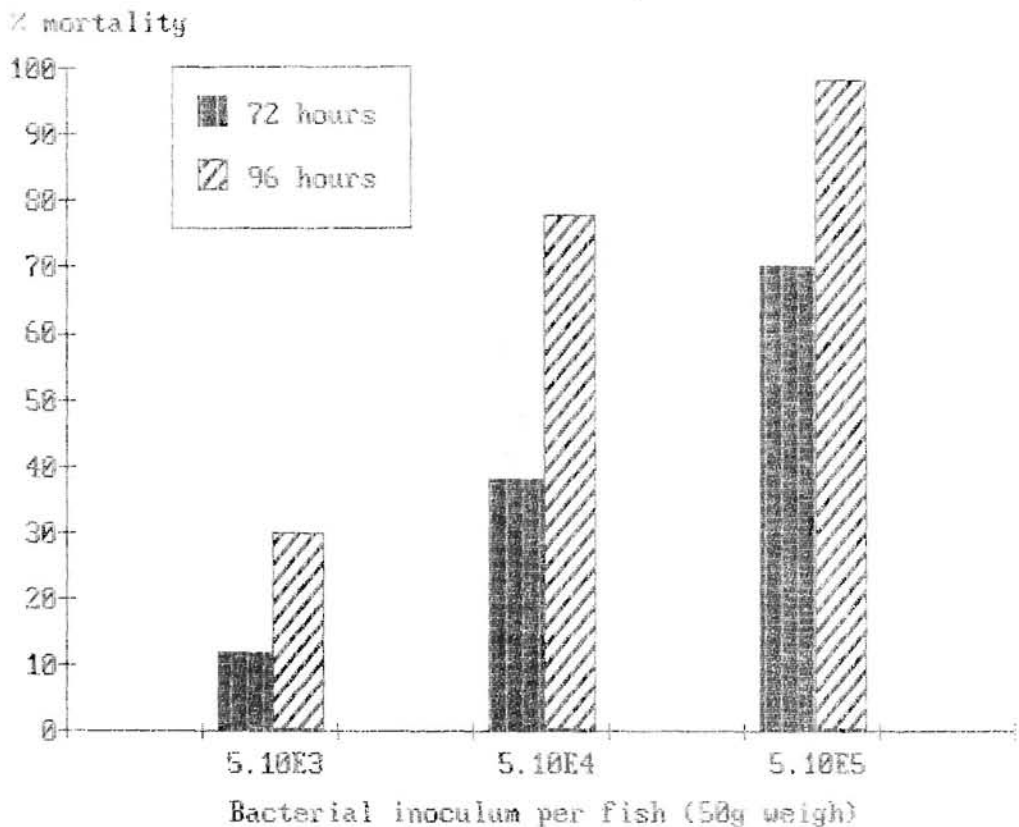


Figure 3. — Percent mortality induced at 72 and at 96 hours by an intraperitoneal inoculation of *V. anguillarum* (V62) to sea bass.

Pathogenicity tests

Intraperitoneal inoculation

Among three different strains tested (*V. anguillarum* V62, *V. parahaemolyticus*, and *V. alginolyticus*), only the V62 induced mortalities within a week with haemorrhagic lesions. *V. anguillarum* V62 is isolated from the blood and kidney of moribond fishes. All the animals which were inoculated with the other strains survived like the control group (IP inoculation with a sterile saline solution). The results of inoculation with the V62 strain including two doses are shown on fig. 3.

Tab. 1. — Biochemical characters of reference strains

V62	V. alg	V. paraph	
Cytochrome oxydase	+	+	+
Nitrate reduction	+	+	+
0/129 sensitivity : 10 µg	+	nd	nd
400 µg	+	+	+
Swarming	- +	-	-
Luminescence	-	-	-
Arginine dihydrolase	+	-	-
Lysine decarboxylase	-	+	+
Ornithine decarboxylase	-	+	+
Growth at 37°C	+	+	+
Growth at 42°C	-	nd	nd
Growth at % NaCL : 0 %	+	-	-
2 %	+	+	+
6 %	+	+	+
8 %	+	+	+
10 %	-	+	-
Voges-Proskauer reaction	+	+	+
Gas from glucose fermentation	-	-	-
Fermentation to acid :			
L-arabinose	-	-	-
m-inositol	-	-	-
D-mannose	+	+	+
Sucrose	+	+	+
Amygdaline	-	-	-
Utilization as sole source of carbon			
Cellobiose	-	-	+
D-gluconate	+	+	+
Sucrose	+	+	+
D-xylose	-	-	-
Enzyme production :			
Alginase	-	-	-
Amylase	+	+	+
Gelatinase	+	+	+
Lipase	+	+	+

nd : not done
V : variable

V62 : *V. anguillarum*
V. alg : *V. alginolyticus*
V. paraph : *V. parahaemolyticus*

Scarification, intradermal and bath challenge

Results are shown on table 2.

An intradermal (ID) inoculation with 10^5 bacteria/fish as well as a 1 hour bath in salt water containing 10^7 bacteria/ml induces mortalities within 3 days. Fishes whose skin had been scarred and exposed to a bacterial suspension of 10^8 CFU/ml, reacted differently depending on the the water temperature of the test. At 20-21°C, fishes which had been stressed by rising the water temperature (group 3) are more sensitive than the others (group 3); moribond fishes do not show ulcerative lesions of the skin. Only fishes scarred at 20°C and then placed at 25°C (group 2) have developed ulcerative lesions of the skin.

Tab. 2. — Scarification, intradermal and bath challenge with the reference strain V62

Bacterial concent.	Test group	Nb	Wgt (g)	Temp (°C)	Temp variat.	Mortality (%)	Skin lesion
10^8 /ml	SC 1	10	15 g	20°		20 %	—
	SC 2	10	15 g	15°	+ 5°	70 %	—
	SC 3	40	15 g	20°	+ 5°	100 %	++
Control	SC	30	15 g	20°	+ 5°	0 %	—

SC = Scarification test

10^5 /anl	ID	6	250 g	20°		50 %	—
control	ID	6	250 g	20°		0 %	—

ID = Intradermal inoculation

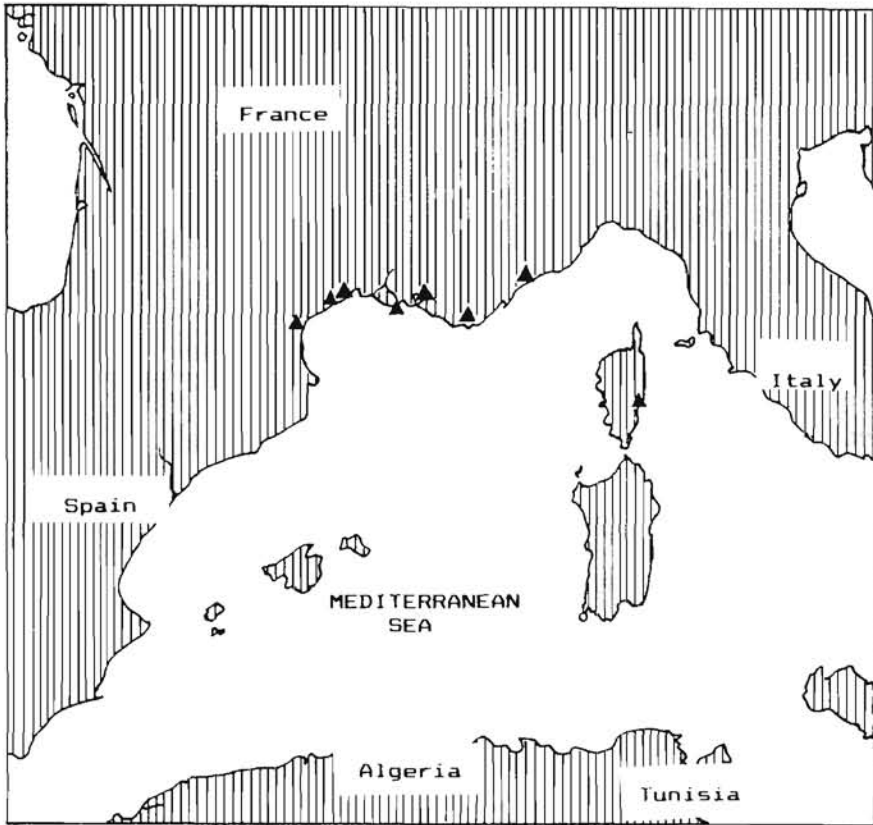
10^7 /ml	1 h	30	1 g	20°		70 %	+
control	12 h	30	1 g	20°		0 %	—

Bath challenge

EPIDEMIOLOGY

Among 70 presumptive *V. anguillarum* V62 strains tested, 59 strains (84 %) are agglutinated by the specific antiserum. However, crossed reactions exist between the two antisera tested (V408 and V62), and suggest antigenic communities between these two strains. Other isolated strains (*Vibrio* sp., *V. alginolyticus*, *V. parahaemolyticus*, *Pseudomonas* sp. and *Enterobacteria* sp.) did not react the V62 antiserum.

Geographical distribution of vibriosis : rearings of sea bass affected with vibriosis are shown on fig. 4.



▲ Mortality with *V.anguillarum* (V62) isolation from diseased fish

Figure 4. — Occurrence of *V. anguillarum* on sea bass from different fishing locations in the South of France.

DISCUSSION

Many marine fish species may develop an ulcerative disease : salmon (Evelyn 1971, Morrisson *et al.*, 1981), Pacific halibut (Levine *et al.*, 1972), cod (Jensen & Larsen, 1982), sea bream (Colorni *et al.*, 1981), grey mullet (Burke *et al.*, 1981), turbot and eel (Colwell and Grimes, 1984). Previous bacterial investigations of diseased animals have involved several genera : *Vibrio* (Burke *et al.*, 1981; Colwell and Grimes, 1984; Colorni *et al.*, 1981; Phelepp and Martin, 1985; Phelepp *et al.*, 1985, Nounou, 1985), *Aeromonas* (Alvarez and Conroy, 1987, Llobrera *et al.*, 1987), *Pseudomonas* (Wakabayashi *et al.*, 1972; Nounou, 1985), *Mixobacteria* (Demoury, 1987). Our bacterial identification may be regarded as a presumptive one; neverthe-

less, *Vibrio anguillarum* has been isolated in different aquatic farms suffering from bacterial disease. The V62 strain differs from the representative French strain of *V. anguillarum* V408 (Baudin Laurencin, 1981) by a few biochemical characters (negative arabinose and amygdaline assimilation, growth on 0% salinity medium). Arabinose negative *V. anguillarum* strains have already been isolated from grey mullet (Burke *et al.*, 1981), catfish (Lewis, 1985) and molluscs (Bolinches *et al.*, 1986); and *V. anguillarum* strains able to grow on a 0% salinity medium have been reported. Pathogenicity tests with the V62 strain have induced mortalities as with other *V. anguillarum* strains inoculated to salmon (Evelyn, 1971), or turbot and trout (Baudin Laurencin, 1986). Experimental challenge with *V. anguillarum* is also known to induce ulcerative lesions on grey mullet (Burke *et al.*, 1981) and on winter flounder (Levine *et al.*, 1972).

Vibriosis is presently the main pathological problem for the rearing of sea bass in France (fig. 4). This pathology may increase in the future as recent data show that trout (Baudin Laurencin, pers. comm) and turbot (Ifremer, Palavas), are also sensitive to the V62 strain. Attempts are made to set up vaccination of the rearings.

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