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ISOLATION OF *PASTEURELLA PISCICIDA* FROM SEA BASS IN SOUTHWESTERN SPAIN

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Pasteurella piscicida is the microorganism responsible for epizootics of wild populations of different fish species, including white perch (*Morone americanus*) (Snieszko *et al.*, 1964), striped mullet (*Mugil cephalus*) (Lewis *et al.*, 1970), and striped bass (*Morone saxatilis*) (Paperna and Zwerner, 1976). However, the pasteurellosis has become more important in fish farms, where this microorganism may cause heavy economical losses. Although the "pseudotuberculosis" is a very old disease in Japan affecting mainly yellowtail (*Seriola quinqueradiata*) (Kubota *et al.*, 1970; Kusuda and Yamaoka, 1972), until recently no reports of this disease were noticed in Europe. Toranzo *et al.* (1991) described the first outbreak of pasteurellosis in Spain which took place in gilthead seabream (*Sparus aurata*) cultured in the North-western area.

In this report we describe the first epizootic of pasteurellosis in sea bass (*Dicentrarchus labrax*) cultured in several farms located in South-western Spain.

The epizootic occurred in juvenile sea bass (average weight 100 g) during September and October 1991. Mortality was observed in a stock of about 200,000 specimens reared in 4 tanks with a density of 100-200 fish/m^3. This outbreak produced a loss of about 15% of the affected stock, with an average mortality rate of 0.05% per day. During the epizootic the water temperature fluctuated between 18° and 20°C; however, the salinity and dissolved oxygen was maintained near constant with values of 35 ppt and 6 ppm, respectively.

For bacterial isolation, samples were taken from spleen, kidney and liver of the moribund specimens, and cultured on Marine agar Z-2216 (Difco) and Thiosulphate Citrate Bile Sucrose (TCBS) agar (Difco). Cultures were incubated at 22°C for 48-72 h.

Pure cultures of the isolated colonies were identified using the standard morphological, physiological and biochemical tests (Carter, 1984; West and Colwell, 1984; Toranzo *et al.*, 1991). The commercial miniaturized API-20E system (BioMerieux) was used in parallel with the conventional biochemical tests. Two reference strains, *P. piscicida* ATCC 17911 isolated from white perch in USA (Snieszko *et al.*, 1964) and DI-21 isolated from gilthead seabream in the Northwest of Spain (Toranzo et al., 1991) were included as controls.

The confirmatory diagnosis of pasteurellosis was conducted by slide agglutination tests (Toranzo *et al.*, 1987) using the antisera raised against the above mentioned two reference strains.

Antimicrobial resistance of the isolates was determined by the disk diffusion method on Mueller Hinton agar (BioMerieux) supplemented with 2% NaCl. The following antimicrobial agents (µg/disk) were tested: ampicillin (10), chloramphenicol (30), kanamycin (30), erythromycin (15), oxytetracycline (30), streptomycin (10), trimethroprim-sulphamethoxazole (25), and nitrofuratoin (300).

Moribund specimens presented a normal external aspect excepting by a swelling in the abdominal cavity. Internally, affected fish showed paleness of liver and kidney, but the typical tubercles on the spleen (Kubota *et al.*, 1970) were not observed. From all these organs large numbers of gram-negative bacilli with a bipolar staining were isolated. Only one type of colony was recovered in pure culture on Marine agar

from the organs examined. No bacterial growth was obtained in the selective medium TCBS.

The biochemical characteristics of the isolates allowed us to identify the bacteria as *Pasteurella piscicida,* since it possesses the same characteristics as the reference strains ATCC 17911 and DI-21 (Table 1). In addition, all the isolates shared the same profile in the API-20 E system (code number: 2005004). The serological assays allowed us to confirm the bacterial identification since the sea bass strains displayed a strong agglutination with the two antisera used.

Table 1. Common characteristics exhibited by the *P. piscicida* strains isolated in the present study and the reference strains.

POSITIVE TESTS	NEGATIVE TESTS
Arginine dihydrolase	Lysine decarboxylase
Lipase	Ornithine decarboxylase
Phospholipase	Indole production
Growth at 10°C and 30°C	Nitrate reduction
Growth in 0.5 and 3% NaCl	Citrate utilization
Acid from:	Urease
ONPG	Amylase
Glucose	Gelatinase
Mannose	Caseinase
Galactose	Haemolysis (sheep blood)
Fructose	Growth at 35°C
Sensitivity to:	Growth in 5% NaCl
Vibriostatic agent O/129	Growth in TCBS
Novobiocin	Acid from: maltose, sucrose, rhamnose, arabinose, amygdalin, inositol, melibiose, mannitol, sorbitol

The sensitivity pattern of our isolates is very similar to those observed for the reference strains, being all the isolates resistant to kanamycin, erythromycin and streptomycin. The oral administration of oxytetracycline (about 40 mg/kg fish per day) to the affected fish population and some baths with nitrofurazone produced a high reduction of fish mortality after 7 days of treatment.

Infectivity trials were carried out to determine the degree of virulence (lethal dose 50%, LD_{50}) of the *P. piscicida* isolates (representative strain B52) for sea bass and gilthead seabream (both with 5 g average body weight). Five fish for each dose were intraperitoneally injected (0.1 ml) with bacterial doses ranging from 10^4 to 10^8 cells per fish. Inoculated fish were maintained in 50 l aquaria at 18°C and 35 ppt of salinity with aeration. Control fish were inoculated with the same volume of sterile saline solution. These assays demonstrated that the inoculated *P. piscicida* strain was pathogenic for both fish species challenged. After 10 days post injection the LD_{50} values were of 2.5×10^5 for sea bass and 1.0×10^6 cells for gilthead seabream. The inoculated strain was reisolated in pure culture from the internal organs (spleen, kidney and liver) of all dead fish. After 20 days post inoculation some survivor fish were sacrificed and we can be able to isolate the pathogen inoculated only from the spleen. No mortalities were recorded during this period on the control population.

Summary

An epizootic outbreak of pasteurellosis in sea bass (*Dicentrarchus labrax*) cultured in southern Spain is described. *Pasteurella piscicida* was isolated in pure culture from the several internal organs of the affected fish, which showed no apparent surface lesions. The infectivity assays revealed that the *P. piscicida* isolate was pathogenic for sea bass and gilthead seabream with LD_{50} of 2.5×10^5 and 1.0×10^6 cells, respectively.

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