Available online at www.sciencedirect.com
ScienceDirect
Aquaculture
Aquaculture 271 (2007) 47–53
www.elsevier.com/locate/aqua-online

Occurrence and virulence of *Pseudoalteromonas* spp. in cultured gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). Molecular and phenotypic characterisation of *P. undina* strain U58

María Jesús Pujalte [a,b,*], Ariadna Sitjà-Bobadilla [c], María Carmen Macián [b,d], Pilar Álvarez-Pellitero [c], Esperanza Garay [a,b,d]

[a] *Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universitat de València, Campus de Burjassot, C/ Dr. Moliner, 50. 46100 València, Spain*
[b] *Departamento de Microbiología y Ecología, Universitat de València, Campus de Burjassot, C/ Dr. Moliner, 50. 46100 València, Spain*
[c] *Instituto de Acuicultura de Torre de la Sal, Consejo Superior de Investigaciones Científicas (CSIC), Torre la Sal s/n, Ribera de Cabanes, 12595 Castellón, Spain*
[d] *Colección Española de Cultivos Tipo (CECT), Universitat de València, Campus de Burjassot, C/ Dr. Moliner, 50. 46100 València, Spain*

Received 14 March 2007; received in revised form 1 June 2007; accepted 17 June 2007

Abstract

During two long-term bacteriological surveys in European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.) culture facilities on the Spanish Mediterranean coast, four different species of *Pseudoalteromonas* were found in the internal organs of animals. *Pseudoalteromonas undina*, *Pseudoalteromonas haloplanktis*, *P. espejiana* and *Pseudoalteromonas* sp. were isolated from 9% out of 177 European sea bass and 11.7% out of 547 gilthead sea bream and they were recovered as pure cultures in 50% of isolations. Among fish with positive bacterial growth, *Pseudoalteromonas* was more prevalent in sea bass showing clinical signs (27.7%) and in gilthead sea bream without clinical signs (22.5%). The possible pathogenicity of seven strains of three *Pseudoalteromonas* spp., selected by their higher frequency in fish with clinical signs, was tested in both sea bass and sea bream. None of them was virulent for gilthead sea bream at any of the assayed doses. Only one strain, – initially isolated from a diseased sea bass and named U58 – was weakly virulent for this fish species, as it killed 100% of experimentally inoculated fish at a dose of 10^7 cfu/fish ($LD_{50} = 1.3 \times 10^7$ cfu/fish). This U58 strain was further characterized as Gram-negative, motile, non-fermenting and non-pigmented rod that requires Na^+ ion for growth. A further phenotypical (including Biolog GN) and phylogenetical (16S rDNA) characterization identified it as *P. undina*. This is the first report on *P. undina* as a pathogen for marine fish.

Keywords: *Pseudoalteromonas undina*; *Dicentrarchus labrax*; Fish pathogenic bacteria; Marine aquaculture; LD_{50}

1. Introduction

European sea bass, *Dicentrarchus labrax*, and gilthead sea bream, *Sparus aurata*, are amongst the most appreciated marine aquacultured fish. The total production

* Corresponding author. Departamento de Microbiología y Ecología, Universitat de València, Campus de Burjassot, C/ Dr. Moliner, 50. 46100 València, Spain. Tel.: +34 963 543 142; fax: +34 963 544 570.
E-mail address: maria.j.pujalte@uv.es (M.J. Pujalte).

0044-8486/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2007.06.015

of both species in 2005 in Europe and the Mediterranean area was more than 170,000 Mt (APROMAR, 2006). The main bacterial pathogens affecting their production have been identified as members of the *Vibrionaceae*, mainly *Vibrio anguillarum* and *Photobacterium damselae* subsp. *piscicida* (formerly '*Pasteurella piscicida*') (Rodgers and Furones, 1998). Other bacteria, such as *Vibrio alginolyticus* and *Vibrio harveyi*, are considered opportunistic pathogens causing mortalities in both fish species in the Spanish Mediterranean area (Balebona et al., 1998; Pujalte et al., 2003b).

Strictly aerobic marine bacteria have been associated with mortality of marine fish in several cases. The most outstanding are *Tenacibaculum maritimum* (formerly *Flexibacter maritimus*), causing mortality in farmed gilthead sea bream and sole (Arijo et al., 2005), European sea bass (Bernardet et al., 1994) and turbot (Alsina and Blanch, 1993), *T. ovolyticum*, a pathogen of eggs and larvae of Atlantic halibut (Hansen et al., 1992), *Pseudomonas plecoglossicida*, the causative agent of bacterial hemorrhagic ascites of ayu (Nishimori et al., 2000), and *Pseudomonas anguilliseptica*, associated to winter disease in gilthead sea bream (Domenech et al., 1999) and reported in several fish species.

The genus *Pseudoalteromonas* was created by Gaulthier et al. (1995) to include 11 species of aerobic marine bacteria formerly classified in the genus *Alteromonas*. An emended description of *Pseudoalteromonas* was further provided by Ivanova et al. (2002). More recently, Ivanova et al. (2004) proposed the new family Pseudoalteromonadaceae to accommodate the genus *Pseudoalteromonas* and the new genus *Algicola*. More than 30 species are included in the relatively heterogeneous *Pseudoalteromonas* cluster, comprising a related group of the so-called non-pigmented species (15 species) and six clusters of pigmented groups (reviewed in Ivanova et al., 2004).

The interest of some *Pseudoalteromonas* spp. is due to their capability of producing biologically active compounds with antibacterial, algicidal, antifungal, agarolytic, cytotoxic and antiviral activities (Holmstrom and Kjelleberg, 1999; Kalinovskaya et al., 2004). Other interesting aspects are related to the probiotic or pathogenic actions of some species. Although several species are associated to marine eukaryotic hosts, only few of them are related to pathologies in these organisms, as *Pseudoalteromonas haloplanktis* in oysters (Colwell and Sparks, 1967), *P. atlantica* in crabs (Costa-Ramos and Rowley, 2004), *P. gracilis*-like in lobsters (Chistoserdov et al., 2005) and *Pseudoalteromonas*-like in scallops (Torkildsen et al., 2005). Association to pathologies in fish hosts is even scarcer, and includes only *P. piscicida* (Bein, 1954), reported as pathogen for fish eggs (Hansen et al., 1992; Nelson and Ghiorse, 1999) and in experimental infections of fish and crustaceans (Euzéby, 2006). The most toxic species seems to be *P. tetraodonis* (syn. *Alteromonas tetraodonis*, *A. haloplanktis*), whose strain GFC isolated from puffer fish (*Fugu poecilonotus*) produces the potent neurotoxin tetraodontoxin, responsible for severe intoxications in humans in Japan and other countries by ingestion of this fish inadequately processed (Euzéby, 2006).

The present study reports on the isolation of several *Pseudoalteromonas* spp. in the framework of epidemiological studies of gilthead sea bream and European sea bass with and without clinical signs. In addition, virulence trials were carried out for seven selected strains and the only virulent one (U58) was phenotypically and phylogenetically characterized.

2. Materials and methods

2.1. Samplings and isolation of the strains

European sea bass and gilthead sea bream cage-cultured on the Spanish Mediterranean coast were sampled along two epidemiological surveys (1999–2000 and 2003–2004). They included periodical samplings of fish without clinical signs, and samplings of fish with clinical signs during episodes of morbidity or mortality. A total number of 547 gilthead sea bream and 177 European sea bass were analyzed. Fish were killed, weighted and measured as previously reported (Pujalte et al., 2003a). Bacteriological analyses from internal organs (head kidney or liver) were performed by inoculating plates of Marine Agar (MA, Scharlab). Incubation was done at 20–25 °C for up to 10 days, and pure cultures were obtained from each type of colony grown (most commonly during the first 24–48 h) by streaking on MA plates. They were maintained both as semisolid stab cultures on marine broth plus 0.3% agar in screw-capped tubes (room temperature), and suspended on marine broth (MB) plus 10% (v/v) glycerol at −70 °C.

2.2. Phenotypic and molecular characterization

An initial phenotypic characterization was done for all the presumptive *Pseudoalteromonas* spp. with a minimal set of phenotypic tests, as previously described (Arias et al., 1999; Pujalte et al., 2003a). In order to obtain a more accurate identification at species level, an extended phenotypic characterization was performed for the strain which resulted to be virulent in the experimental infections. It consisted of the determination of additional carbon and energy sources and hydrolytic activities, plus the oxidation