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Pathogenesis of *Photobacterium damselae* subspecies infections in sea bass and sea bream

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ABSTRACT

Photobacterium damselae is a Gram negative bacterium causes photobacteriosis, a worldwide septicemic disease in aquaculture including sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). The pathogenicity of bacterial subspecies and the disease pathological changes in natural and experimental infections have thus far yielded inconsistency of effective preventive measures. This study aimed to represent a comprehensive analysis of the potential pathogenic capacities of the two subspecies of *P. damselae* in cultured sea bass and sea bream in the Northwestern region of Egypt. Diseased 321 sea bass and 257 sea bream, in addition to 99 healthy sea bass fingerlings were sampled from three farms located along the Mediterranean Sea. *P. damselae* subspecies were isolated from diseased fish and characterized using bacteriological, molecular, and antimicrobial susceptibility methods. Healthy fish were challenged by a virulent *P. damselae* subsp. *piscicida*, monitored for disease signs and mortality, and the histopathological abnormalities and hematological disorders were carried out. Clinical signs and gross lesions in naturally infected sea bass and sea bream showed great similarities with absence of a subspecies-specific characteristic sign or lesion. The two subspecies were recovered through the entire year from individual fish sample, suggests a coexistence of two subspecies endemic infection. In diseased sea bass, 38.32% and 16.20% were positive for *P. damselae* subsp. *piscicida* and subsp. *damselae*, respectively. However in diseased sea bream, 44.47% and 26.46% were positive for *P. damselae* subsp. *piscicida* and subsp. *damselae*, respectively. High mortalities and devastating clinicopathologic abnormalities represented by sever clinical signs, hematological disorders and histological abnormalities strengthen the pathogenicity of *P. damselae* subspecies in the two fish species and therefore, a vaccination strategy against both subspecies should be taken into account.

1. Introduction

Aquaculture plays an increasingly important role in food production worldwide. However, disease outbreaks have direct effects on fish production, causing severe economic losses in the aquaculture sector. The emergence of bacterial pathogens in aquaculture systems has been a subject of great interest to microbiologists [1]. Currently, the species *Photobacterium damselae*, a member of the genus *Photobacterium* of the Vibrionaceae family, comprises two subspecies, *P. damselae* subsp. *damselae* and *P. damselae* subsp. *piscicida* [2]. *P. damselae* subsp. *piscicida* is a primary pathogen responsible for acute fish photobacteriosis (pseudotuberculosis), a very serious bacterial septicaemia in mariculture worldwide due to its wide host range, massive mortality, ubiquitous distribution, widespread antibiotic resistance and lack of efficient vaccines [3]. *P. damselae* subsp. *piscicida* is able to infect a wide variety of marine fish, including the yellowtail (*Seriola quinqueradiata*) in Japan, gilthead sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), and sole (*Solea senegalensis* and *Solea solea*) in Europe, striped bass (*Morone saxatilis*), white perch (*Morone americana*), and hybrid striped bass (*Morone saxatilis* × *Morone chrysops*) in the USA, cobia (*Rachycentron canadum*) in Taiwan, and golden pompano (*Trachinotus ovatus*) in China [4–6]. *P. damselae* subsp. *damselae* has been recognized as an opportunistic pathogen for a wide variety of fish and mammalian species including human [7]. These strains cause septicemia in warm and cold water fish such as damselfish (*Chromis punctipinnis*), eels (*Anguilla anguilla*), brown shark (*Carcharhinus*

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plumbeus), yellowtail (*Seriola quinqueradiata*), sea bream and turbot (*Scophthalmus maximus*) [8–10].

The diagnosis and differentiation of infections caused by any of the two closely related species of *P. damselae* are carried out using standard microbiological methods (culturing, isolation and biochemical steps) [3]. The miniaturized system AIP20E is usually used for a presumptive identification of the *P. damselae* subsp. *piscicida*. Molecular methods have been developed in order to achieve an accurate and specific identification of *P. damselae* subsp. *piscicida* and a rapid diagnosis of photobacteriosis. A multiplex PCR method based on the 16S rRNA and *ureC* genes has been developed to overcome the limitations of the conventional microbiological techniques and to allow the discrimination between the two subspecies. The *ureC* gene is present in *P. damselae* subsp. *damselae* genome but is not found in *P. damselae* subsp. *piscicida* [11].

Although antibiotics have been the first line of defense in fish aquaculture to control photobacteriosis outbreaks, *P. damselae* subsp. *piscicida* was reported to be sensitive to some antibiotics such as chloramphenicol, tetracycline and ampicillin [7]. However, the extensive use of these antibiotics has resulted in the appearance of resistant bacteria [8]. Moreover, different transferable genetic elements (R plasmids) carrying genes for resistance against many antibiotics have been documented in *P. damselae* subsp. *piscicida* [3] and so several studies have attempted to develop vaccines against photobacteriosis.

Understanding the occurrence and spread of bacterial pathogens is essential for managing the health and welfare of the cultured fish and ensuring the success of mariculture [12]. Multidisciplinary studies involving the virulence factors of the pathogenic microorganisms, aspects of the biology and immunology of the fish, as well as a better understanding of the environmental conditions affecting fish cultures, will allow the application of adequate measures to control and prevent the microbial diseases limiting the production of marine fish [13]. To date, little information is available regarding the pathogenicity of the two *P. damselae* subspecies and the overall biological changes associated with the infection in many fish species under natural conditions and even under experimental conditions. Previous studies showed that different genotypes of *P. damselae* subspecies *piscicida* and *damselae* can have different phenotypic characteristics and pathogenicity potential [14]. Therefore, the evaluation of the occurrence of distinct populations within a subspecies and understanding the pathogenicity during the course of infection under natural and experimental conditions can be important for proper assessment of each case and effective disease control. This facilitates proper management and accurate diagnosis, and provides essential information needed for the development of an effective vaccine against photobacteriosis. Sea bass and sea bream are the predominant and most widely cultured fish species in the countries of the Mediterranean Basin, including Egypt [15]. The intensive culture of these fish species has favored the appearance of several outbreaks with varied mortality rates. Although the current data available on the bacterial pathogens of sea bass and sea bream are limited and controversial [16], mostly they are the common two fish species susceptible to photobacteriosis [17]. Therefore, there is a necessity to evaluate the impact of two subspecies of *P. damselae* to aquaculture in Egypt and improve our understanding of the interaction between this bacteria and fish. The objective of the present study was to study the pathogenicity of the two subspecies of *P. damselae* in diseased sea bream and sea bass with a mass mortality. Additionally, attention has been focused on the potential pathogenic capacities of virulent strain of *P. damselae* subsp. *piscicida* isolated from natural outbreak, on challenged sea bass. The obtained results provide some essential information regarding the pathogenicity of the two subspecies of *P. damselae* that might enrich the effective preventive measures against photobacteriosis.

2. Materials and methods

2.1. Fish collection and sampling

A total of 578 moribund and surviving fish (321 sea bass and 257 sea bream) were sampled between January and December 2014 from three farms chosen to represent the Egypt's Northwest region located along the Mediterranean Sea (Table 1): Elmax hatchery; Wadi Mariout Lake; and ELKilo 21 hatcheries. A preference for sample selection was made for fish demonstrating signs of disease and the disease clinical signs in all fish samples were recorded. Alive fishes were put in plastic tanks supplied with air blower and the recently dead samples were put in ice boxes and sent to the Fish pathology laboratory, National Institute of Oceanography and Fisheries, (NIOF), for bacteriological, molecular and antibiogram tests. In addition, a total of 99 healthy sea bass fingerlings, which had no history of disease, in Elmax hatchery were sampled for the challenge test.

2.2. Disease signs and gross lesions

At the laboratory: Each sampled fish was surface disinfected with 0.1% benzalkonium chloride saline solution (BZC) and thorough rinsing with sterile phosphate-buffered saline complemented with 1.5% sodium chloride (NaCl) at pH 7.2 (PBSS). The clinical signs and physical abnormalities were reevaluated and recorded. Each fish underwent a full postmortem examination for gross pathological signs, as previously described [18]. In particular, gross lesions in gills, liver, kidney and spleen were examined and photographed.

2.3. Bacterial isolation and culture conditions

The detection of *P. damselae* and distinguishing the isolates at the subspecies level were achieved using the standard procedures of morphological, physiological and biochemical plate and tube tests as described in Bergey's Manual of Determinative Bacteriology [19]. Briefly, the gills and internal organs (livers, spleens, kidneys, and hearts) of anaesthetized fish were completely removed and homogenized in 2 ml of sterile PBSS. The homogenate was then diluted serially10-fold and an aliquot (0.1 ml) of dilution was plated in duplicate on a brain–heart infusion agar (BHIA; Oxoid) supplemented with 1% NaCl and incubated at 25 °C for 1–2 days. To differentiate *P. damselae* subsp. *piscicida* from *P. damselae* subsp. *damselae* [20], fresh colonies were picked at random from the BHIA plates and inoculated on the surface of two different plates containing thiosulphate citrate bile salt sucrose agar (Oxoid) supplemented with 1.5% NaCl (TCBS-1) and 5% sheep blood agar supplemented with 3% NaCl. The plates were incubated at 25 °C for 24 h. *P. damselae* subsp. *damselae* grows on TCBS-1 producing green colonies whereas *P. damselae* subsp. *piscicida* does not grow [21]. *P. damselae* subsp. *damselae* exhibits hemolytic activity on blood agar plates whereas *P. damselae* subsp. *piscicida* lacks observable hemolytic activity [22]. All colonies having dissimilar visual appearances were sub-cultured from a single colony for purity. Pure presumptive colonies isolated on plating media were used for phenotypic and biochemical analysis including the following tests: Gram staining, oxidase, lysine decarboxylase production (LDC), ornithine decarboxylase (ODC), β –galactosidase, motility in marine broth plus 0.4% agar, nitrate reduction to nitrite, gas production from glucose and urease production, indole production in marine broth (MB), and fermentation of mannitol, melibiose and amygdalin. A commercial miniaturized API 20NE Kit (Bio-Mérieux, Inc)