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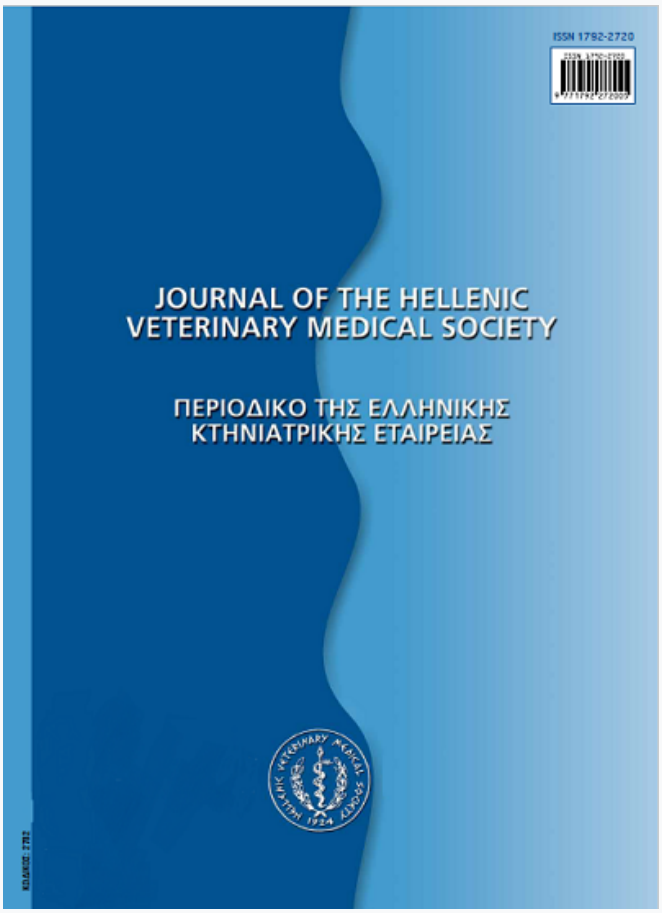

Tenacibaculosis in aquaculture farmed marine fish

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Review article
Ανασκόπηση

Tenacibaculosis in aquaculture farmed marine fish

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Η Μυξοβακτηριδίαση στις ιχθυοκαλλιέργειες θαλάσσιων ψαριών

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ABSTRACT. Tenacibaculosis is a limiting factor of the culture of many farmed marine fish worldwide. In marine fish species, the main etiological agent of the disease is the bacterium Tenacibaculum maritinun. The disease is responsible for high mortalities in intensive aquaculture farms. The infection can cause external pathological signs and lesions to the fish, such as ulcers, hemorrhagic and necrotic lesions on the skin, fins and tail, hemorrhagic stomatitis and corrosion of the tail and fins. In the present review information is provided regarding Tenacibaculum maritinun strains that infect farmed marine fish, the disease, the causative agent, host species, clinical symptoms, methods of diagnosis, pathogenesis of infection, the treatment and prevention of the disease.

Keywords: *aquaculture, farmed marine fish, Tenacibaculosis, Tenacibaculum maritinun*

ΠΕΡΙΛΗΨΗ. Η μυξοβακτηριδίαση αποτελεί περιοριστικό παράγοντα εκτροφής πολλών θαλασσινών ψαριών παγκοσμίως. Στα θαλασσινά είδη ψαριών, το κύριο αίτιο της νόσου είναι το βακτήριο Tenacibaculum maritinun. Η νόσος, είναι υπεύθυνη για μεγάλες θνησιμότητες στις εντατικές ιχθυοκαλλιέργειες. Η μόλυνση μπορεί να προκαλέσει εξωτερικά παθολογικά συμπτώματα και αλλοιώσεις στα ψάρια, όπως έλκη, αιμορραγικές και νεκρωτικές αλλοιώσεις στο δέρμα, πτερύγια και ουρά, αιμορραγική στοματίτιδα και διάβρωση της ουράς και των πτερυγίων. Στην παρούσα ανασκόπηση αναφέρονται πληροφορίες για τα στελέχη Tenacibaculum maritinun που προσβάλλουν εκτρεφόμενα ψάρια του θαλασσινού νερού, για την νόσο, τον αιτιολογικό παράγοντα, τους ξενιστές, την κλινική εικόνα, τις μεθόδους διάγνωσης, την παθογένεια της μόλυνσης, την θεραπεία και την πρόληψη της νόσου.

Λέξεις ευρετηρίασης: *ιχθυοκαλλιέργειες, θαλάσσια εκτρεφόμενα ψάρια,* Μυξοβακτηριδίαση, *Tenacibaculum maritinun*

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INTRODUCTION

Aquaculture in the last four decades, has been one of the fastest growing industries and has managed to contribute today 50% on the total fish consumption worldwide (FAO, 2012). This reflects the vitality of the aquaculture sector in the global economic growth (FAO, 2009). In Greece, aquaculture also provides a significant contribution to primary sector production, with high exports of quantity produced (FEAP, 2014).

In a period where aquaculture is growing fast, one of the major limiting factors of economic stability are the diseases of fish, like the disease of tenacibaculosis from the bacterium *Tenacibaculum maritimun*.

Among the cultured fish, the disease has been reported in marine fish species, like red sea bream (*Pagrus major*), gilthead seabream (*Sparus aurata*),european sea bass (*Dincentrarchus labrax*), atlantic salmon (*Salmo salar*), sole *(Solea solea),* turbot (*Scophthalmus maximus*) and many other cultured and wild fish in Europe, Japan, North America, Australia and all over the world (McVicar and White, 1979,1982; Wakabayashi et al., 1986; Devesa et al., 1989; Pazos et al., 1993; Chen et al., 1995; Handlinger et al., 1997; Ostland et al., 1999; Santos et al., 1999; Avendaño-Herrera et al., 2004a, Choi et al., 2006; Jung et al., 2006; Sheu et al., 2007;Vatsos, 2007; Heindl et al., 2008; Wang et al., 2008; Piñeiro-Vidal et al., 2008a,b; Lee et al., 2009; Oh et al., 2012; Piñeiro-Vidal et al., 2012; Failde et al., 2013).

The affected fish have haemorrhagic mouth, ulcerative skin lesions, hemorrhagic and necrotic lesions on the skin, fins and tail, frayed fins and tail rot, necrosis on the gills and mortality that can reach 20-30%. A systemic disease can be also established involving different internal organs (Magariños et al., 1995; Pazos et al., 1996; Santos et al., 1999; Avendaño-Herrera et al., 2004a; Toranzo et al., 2005).

The disease is worldwide known, causing high mortalities in aquaculture (Santos et al., 1999; Salati et al., 2005; Toranzo et al., 2005; Avendaño-Herrera et al., 2006; Vatsos, 2007; Kolygas et al., 2012; Gourzioti, 2014). In a recent research that was performed in 2011-2013, the presence of the bacterium *T. maritimun* was investigated in farmed marine fish in Greece and was isolated from seven different, farmed marine fish: *Dicentrarchus labrax*, *Sparus aurata*, *Pagellus erythrinus*, *Pargus pargus*, *Umbrina cirrosa*, *Diplodus puntazzo*, *Sciaena umbra* (Kolygas et al., 2012; Gourzioti, 2014).

"Myxobacteriosis" is a term used to denote a group of bacterial infections affecting both freshwater and marine fish, causing disease. There are three main diseases described in the literature: "Rainbow trout fry syndrome", "Bacterial cold water disease/saddleback syndrome" and "Tenacibaculosis/Eroded mouth syndrome". The two first conditions refer mainly to salmonids and other freshwater fish and are caused by *Flavobacterium psychrophilum* and *Flavobacterium columnare.* The third one affects marine fish and is caused by *T. maritimun* (Dipnet report, 2007).

Tenacibaculosis is also referred as marine tenacibaculosis, flexibacteriosis, salt water columnaris disease, gliding bacterial disease of sea fish, EMS (eroded mouth syndrome), BPN (black patch necrosis), Myxobacterial disease, peduncle disease, Saddleback, Fin rot και Cotton wool Disease (Santos et al., 1999; Noga, 2000; Toranzo et al., 2005).

CAUSATIVE AGENT

Tenacibaculum maritimun (formerly *Cytophaga marina, Flexibacter marinus* and *Flexibacter maritimus*) a Gram-negative bacterium, is the etiological agent of tenacibaculosis in marine fish (Wakabayashi et al., 1986; Devesa et al., 1989; Bernardet and Grimont, 1989; Chen et al., 1995; Pazos et al., 1996; Handlinger et al., 1997; Ostland et al., 1999; Santos et al., 1999; Suzuki et al., 2001). Microscopic, the bacterium forms long and slender rods (0.5 μm by 2-30 μm), and occasionally filaments up to 100 μm in length, depending on the culture (Fig. 1). In older cultures, cells appear shorter and tend to become spherical. The bacterium is obligatory aerobic, microcysts are absent and appears a characteristic gliding motility (Pazos et al., 1996; Santos et al., 1999).

The bacterium was first isolated from juvenile red sea bream (*Pagrus major*) in Japan, in 1979 (Hikida et al., 1979) and characterized by Wakabayashi et al. (1986), who validated the species under the name *Flexibacter maritimus*. The first report of the bacterium in Europe was made in 1982, in Dover sole (*Solea solea*) (Campbell and Buswell, 1982; Bernardet et al., 1990). In 2001, bacteria *F. maritimus, F. amylolyticum, F. ovolyticum, F. mesophilum, F. skagerrakense*and *F. lutimaris* were transferred to the new genus *Tenacibaculum* (Suzuki et al., 2001), based on the nucleotide sequence of the gene *gyrB*. The genus *Tenacibaculum* (Suzuki et al., 2001), belongs to the family *Flavobacteriaceae* and today includes the species: *T. adriaticum* (marine bryozoan), *T. aestuarii* (tidal flat sediment), *T. aiptasiae* (sea anemone), *T. amylolyticum* (marine macroalgae), *T. crassostreae* (pacific oyster), *T. dicentrarchi* (European sea bass), *T. discolor* (Dover sole), *T. gallaicum* (turbot), *T. japonica*, *T. litopenaei* (shrimp mariculture pond), *T. litoreum* (tidal flat sediment), *Tenacibaculum lutimaris* (tidal flat sediment), *Tenacibaculum maritimun* (diseased marine fish), *T. mesophilum* (sponges), *T. ovolyticum* (fish eggs), *T. skagerrakense* (seawater), *T. soleae* (Dover sole) *T. jejuense* (offshore seawater) (Wakabayashi et al.,1986; Hansen et al., 1992; Suzuki et al., 2001; Frette et al., 2004; Yoon et al., 2005; Choi et al., 2006; Jung et al., 2006; Sheu et al., 2007; Heindl et al., 2008; Wang et al., 2008; Piñeiro-Vidal et al., 2008a,b; Lee et al., 2009; Oh et al., 2012; Piñeiro-Vidal et al., 2012).

HOST SPECIES

In Europe, *T. maritimun* has been isolated from various fish species: *Solea solea, Solea senegalensis, Dicologogloss cuneata, Dicentrarchus labrax, Sparus aurata*, *Mugil cephalus, Pagrus major, Pagrus pagrus, Puntazzo puntazzo*, *Dentex dentex*, *Umbrina cirrosa, Chelidonichthys lucernus, Scophthalmus maximus, Salmo salar* L. and *Oncorhynchus kisutch*. In Japan, infections have been recorded to *Pagrus major, Acanthopagrus schlegeli, Paralichthys olivaceous, Seriola quinqueradiata, Diplodus sargus* and *Diplodus puntazzo*. In America, the bacterium has been isolated from *Atractoscion nobilis, Sardinops sagax, Engraulis mordax* and *Oplegnathus fasciatus*. In Australia, the main fish species that can be affected are *Salmo salar* and *Oncorhynchus mykiss*. Today the disease cause severe loses in marine aquaculture (Baxa et al., 1986; Wakabayashi et al.,1986; Bernardet et al., 1990; Chen et al., 1995; Soltani et al.,1996; Santos et al., 1999; Cepeda and Santos, 2002; Salati et al., 2005; Toranzo et al., 2005; Avendaño-Herrera et al., 2004b,2005b; Magi et al., 2006; van Gelderen et al., 2009). *T. maritimun* has also been isolated from aquarium fish: Picasso Tigger Fish (*Rhinecanthus assasi*) and Black damsel fish (*Neoglyphieodon meles*), causing lesions and mortality (Mohamed et al., 2011).

SEROLOGICAL STUDIES

Until 1999, *T. maritimun* was considered biochemical and serological homogeneous taxon (Ostland et al., 1999). Recent studies, using serological and molecular methods, have proved the presence of serogroups in the species (Pazos, 1997; Santos et al., 1999; Ostland et al., 1999). Serologically, at least three groups of *T. maritimun* isolates from strictly marine fish can be distinguished (Avendaño-Herrera et al.,2004a). These groups are associated with the host origin; specific fish species are affected with specific serogroup of the pathogen and vice versa (Avendaño-Herrera et al., 2004a).

Dot blot assays and immunoblot analysis of lipopolysaccharides (LPS) revealed the existence of antigenic diversity in *T. maritimun* and demonstrated that at least 3 major O-serogroups seemingly related to the host species can be detected (Avendaño-Herrera et al. 2004b, 2005b). Thus, the majority of *T. maritimun* isolated from sole in the northwest of Spain and all gilthead sea bream isolates belonged to serotype O1, while all strains isolated from sole in Portugal and southern Spain constituted a serotype (O3), different from those strains isolated from turbot (serotype O2). However, these serological differences could certainly be extended if further studies, including more strains of *T. maritimun* isolated from different hosts and/or

geographical origins (Avendaño-Herrera et al., 2004a).

The first serological studies described by Wakabayashi et al. (1984) and Pazos et al. (1993), reported antigenic homogeneity of *T. maritimun*, regardless of their origin and source of isolation. Further studies by Pazos (1997) and Ostland et al. (1999) demonstrated antigenic differences among *T. maritimun* isolates, suggesting that this microorganism may not be as homogeneous as previously thought. Further studies are necessary, since a clear definition of antigenic knowledge of this bacterium is of crucial importance for the development of an effective vaccine (Avendaño-Herrera et al., 2004a; Romalde et al., 2005).

CLINICAL SIGNS

The affected fish (juveniles or adults) have eroded mouths, ulcerated and hemorrhagic skin lesions, frayed fins, necrosis of the caudal fin and tail rot (Fig. 2-3) (Magariños et al., 1995). Adult fish or juveniles can be affected, although younger fish seems to be more sensitive and suffer a more severe form of the disease. High water temperature, above 15°C (Pazos et al., 1996; Santos et al., 1999), stress and the condition of skin surface influence the emergence of the disease (Toranzo et al., 2005).

In juveniles, hemorrhagic stomatitis and eroded mouth (Fig. 4), that is covered with pale yellow mucus layer can be observed (Avendaño-Herrera

Figure 1. Long and slender rods *Tenacibaculum maritimun* in smears (x1000 objective).

Figure 2. Sea bass affected by tenacibaculosis. Pale yellow lesion and necrosis of the caudal fin.

Figure 3. Sea bass affected by tenacibaculosis. Necrosis and fray of the caudal fin.

Figure 4. Sea bass affected by tenacibaculosis. Hemorrhage mouth, haemorrhage stomatitis.

et al., 2004a). Infected tissue can appear vale yellow due to the presence of large number of bacteria. Some affected fish may have shallow skin lesions or darkening of tissue between caudal and marginal fin rays followed by extensive darkening of the area, loss of epithelial surface, and hemorrhage in exposed dermal tissue. Hemorrhagic jaws have also been observed in some fish. In some cases, fish can have necrosis on the gills and increased mucus (Pazos et al., 1996; Santos et al., 1999). A systemic disease can be also established, not often, involving different internal organs.

Depending on the virulence of the strain, acute or chronic disease can be observed. In acute disease usually fish don't appear any clinical sign and only mortality can be observed (Schaperclaus, 1992).

The loss of epithelial fish surface, typical of this disease, is also a portal of entry for other bacterialor parasitic pathogens, causing mixed infections. The lesions favor the entrance of other pathogenic bacteria (Vibrio spp.) and saprophytic organisms such as ciliated protozoans (Devesa et al.,1989; Avendaño-Herrera et al., 2004a).

DIAGNOSIS

The clinical signs, along with the microscopical observation of long rods in wet mounts or Gram-stained preparations obtained from gills or lesions (Fig. 5), can be used as the initial step for the diagnosis of the disease (Toranzo et al., 2005). However, the difficulty of finding *T. maritimun* in early lesions and the high incidence of secondary bacterial infections, makes diagnosis difficult and increases the possibility of misdiagnosis (Hikida et al., 1979; Handlinger et al., 1997).

For the confirmation of the initial diagnosis, cultivation and isolation of the bacterium in the appropriate media, examination of its biochemical profile and molecular diagnosis with DNA-PCR methology from skin samples can be used. Diagnosis may also be supported by serological methods such as fluorescent antibody techniques (Santos et al., 1999; Suzuki et al., 2001; Avendaño-Herrera et al., 2004a).

Isolation-culture

T. maritimun only grow in specific media since it needs an absolute requirement of seawater as well as low concentration of nutrients (Toranzo et al., 2005). The isolation of the bacterium in general nutrient substrates is difficult, because of the slow growth and overgrowth of other heterotrophic bacteria. Several media, including:Anacker & Ordal agar (AOA) (Anacker and Ordal, 1959) or variations of this agar, prepared with 70% seawater (Bullock et al., 1986; Santos et al., 1999), *Flexibacter maritimus* medium (FMM) (Pazos et al., 1996), Selective Flexibacter medium (SFM) or Hsu and Shotts medium that contains neomycin (Bullock et al., 1986; Chen et al., 1995), Marine Agar (MA) (Frerichs, 1993) and Hsu-Shotts medium prepared with 50% seawater (Chen et al.,1995), have been used for the isolation of *T. maritimun*. Additionally, different specific substrates that contain antibiotics (neomycin, flumequine) have been used for the isolation of the bacterium (Bullock et al., 1986). FMM proved to be the most effective for the recovery of this pathogen from fish tissues (Pazos et al., 1996; Avendaño-Herrera et al., 2006).

Incubation is usually carried out at 20^0C–25^0C for 48-72 h. On FMM and AOA media, typical colonies of the bacterium are pale-yellow, flat, with uneven edges and strongly adherent to the medium (Fig. 6), while on Marine agar, colonies are round and yellow pigmented. In static liquid medium, surface growth is in the form of a pellicle (Pazos et al., 1996; Santos et al., 1999; Avendaño-Herrera et al., 2004c). No growth occurs in medium prepared with salt (NaCl) instead of seawater, while *T. maritimun* requires two inorganic salts (KC1 and NaCl) for growth (Santos et al., 1999).

Biochemical identification

Biochemical profile of *T. maritimun* remains an important step for the final diagnosis and is based on the homogeneity of the biochemical properties of *T. maritimun* (Wakabayashi et al., 1986; Bernardet and Grimont, 1989; Chen et al., 1995). Biochemical identification is based on the use of commercial biochemical trials and miniaturized systems, such as API

20E, API ZYM, API 50CH (Bernardet and Grimont, 1989; Pazos et al., 1993; Bernardet et al., 1994; Chen et al., 1995; Ostland et al., 1999; Avendaño-Herrera et al., 2004b). Concerning the API ZYM system, the majority of isolates display positive results in the first 11 enzymatic reactions while the strains don't degrade carbohydrates.

On solid media, *T. maritimun* colonies absorb Congo red. Produces enzymes that degrade casein, tyrosine and tributyrin, but it does not hydrolyse agar, carboxymethyl cellulose, cellulose, starch, esculin or chitin (Santos et al., 1999). Variable results have been reported for gelatin, hydrogen sulfide and nitrate reactions (Bernardet et al., 1990; Chen et al., 1995; Ostland et al., 1999; Avendaño-Herrera et al., 2004a). As suggested by Suzuki et al. (2001), the employment of different basal media could account for this variability. Catalase and oxidase are positive in all strains. In addition, growth occurs on tryptone, casamino acids and yeast extract as the sole carbon and nitrogen source (Avendaño-Herrera et al., 2004a).

Molecular identification

The application of the PCR methodology is important for accurate identification of the pathogen, replacing the phenotypical and biochemical diagnostic methods (Toranzo et al., 2005). PCR methodology remains a powerful tool for an accurate identification of the pathogen from plate cultures as well as from tissues, allowing the production of large numbers of copies of a particular DNA sequence (Avendaño-Herrera et al., 2004a).

Until today, two PCR primer pairs have been designed for the detection of *T. maritimun* using the 16S rRNA gene as target (Toyama et al., 1996; Bader and Shotts et al., 1998). Toyama et al. (1996) selected a pair of primers MAR1 (5′-AATGGCATCGTTTTAAA-3′) and MAR2 (5′-CGCTCTCTGTTGCCAGA-3′), positions 190 to 206 and 1262 to 1278, respectively, in the *Escherichia coli* 16S rRNA numbering system, flanking a 1088 bp fragment. Bader and Shotts (1998) also selected a pair of *T. maritimun* species-specific PCR primers Marl (5′-TGTAGCTTGCTACAGATGA-3′) and Mar2 (5′-AAATACCTACTCGTAGGTACG-3′), positions 77 to 98 and 1060 to 1081, respectively, from unique sequence stretches within this gene, delimiting a 400 bp DNA fragment.

A comparative evaluation of the specificity and sensitivity of both methods (Avendaño-Herrera et al., 2004c) demonstrated that the Toyama PCR protocol was the most adequate for the accurate detection of *T. maritimun* in diagnostic pathology as well as in epidemiological studies of tenacibaculosis.

In order to increase its sensitivity, a nested PCR approach was developed and was evaluated in experimentally seeded fish tissues and in field studies

Figure 5. Gram negative, long rods T. maritimun smears (Gram stain, x1000 obgective).

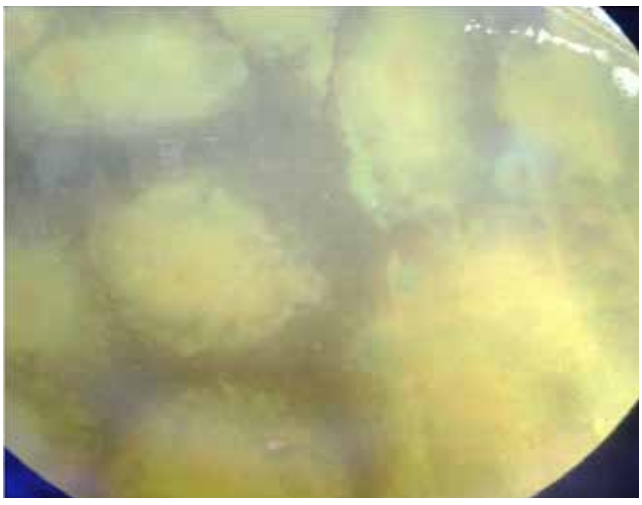

Figure 6. Yellow colonies, with uneven edges of T. maritimun, from the skin on FMM agar.

(Avendaño-Herrera et al., 2004a,c). The high level of *T. maritimun* found in mucus samples (10^3cfu/ml) indicates that this non-destructive method is very useful for a specific and rapid (7 h) diagnosis of tenacibaculosis.

PATHOGENESIS OF INFECTION

Despite the significance of *T. maritimun* in the aquaculture industry, relatively little is known about the pathogenicity of this bacterium. The mode of transmission and the route of infection of *T. maritimun* are not well understood. Strains of the bacterium can survive in the aquatic environment for long time (Roberts, 2001). The natural reservoirs of the pathogen are unknown, but the microorganism can be isolated from sediments and water that have been exposed to infected stocks (Wakabayashi et al., 1984; Soltani et al., 1996; Santos et al., 1999; Powell et al. 2004; Salati et al., 2005; Choi et al., 2006; Jung et al., 2006). Transmission through sea water and direct transmission from host to host have been proposed as possible routes, in addition to ingestion along with food (Mitcell and Rodger, 2011). Also, different species of jellyfish and sea lice seems to act as vectors for the bacteria (Ferguson et al., 2010).

It is well known that the primary sites of infection with *T. maritimun* are body surfaces such as the head, mouth, fins, and flanks. This pathogen attaches itself strongly to the external skin and mucus of fish which do not contain compounds that inhibits the growth of this bacterium (Magariños et al., 1995). The localization of the bacteria within the mucus layer suggests that *T.maritimun* could be part of the autochthonous populations of the fish skin, and therefore the pathogen can remain in the aquatic environment for a long time, utilizing fish mucus as a reservoir (Avendaño-Herrera, 2005b).

T. maritimun shows a lack of strict host specificity. Therefore, tenacibaculosis can be a risk for many species of anadromous and marine fish in which the disease has not been yet described. Although both adults and juveniles may be affected by the disease, younger fish with body weights ranging from 2 to 80 g suffer the highest occurrence and fish above 100 g appear to be resistant (McVicar and White, 1979; Wakabayashi et al., 1984; Bernardet et al., 1994; Handlinger et al., 1997; Avendaño-Herrera, 2006). This is due to the apparently greater susceptibility of smaller fish to *T. maritimun* (Bernardet et al., 1994).

An increased prevalence and severity of the disease has been reported at higher temperatures (above 15°C) and salinities (30 to 35‰) as well as with low water quality. However, winter outbreaks of tenacibaculosis have also been reported (Wakabayashi et al., 1984; Bernardet et al., 1994; Soltani et al., 1996). In addition to these factors, the disease is influenced by a multiplicity of environmental conditions (stress), management factors (high density, poor feeding, handling of fish) and host-related factors (skin surface condition) (McVicar and White, 1979; Wakabayashi et al.,1984; Chen et al., 1995; Magarinõs et al., 1995; Handlinger et al., 1997). In these conditions, the systemic disease involving different internal organs became more prevalent (Alsina and Blanch, 1993; Cepeda and Santos, 2002; Avendaño-Herrera et al., 2004b), indicating that *T. maritimun* has strong virulence mechanisms.

TREATMENT

The appearance of outbreaks can be avoided or the incidence of the disease can be reduced by controlling fish densities (avoiding overcrowding), reducing stress conditions, removing parasites, improving quality of water and avoiding overfeeding (Santos et al., 1999).

Studies on the susceptibility of *T. maritimun* to various chemotherapeutic agents indicate that bacterial strains isolated from different host species and geographical regions exhibit a similar pattern, with respect to susceptibility to nitrofurans, penicillins, amoxycillin, erythromycin, tetracyclines, oxytetracycline, chloramphenicol, trimethoprim, potentiated sulfonamides and fluoroquinolones, and resistance to colistin, kanamicyn, neomicyn and the quinolones, oxolinic acid and flumequine (Baxa et al., 1988c; Pazos et al., 1993; Chen et al., 1995; Handlinger et al., 1997; Santos et al.,

1999; Avendaño-Herrera et al., 2004b, 2005a, 2006, 2008). Also, most of the treatments that are used are based on the use of antibiotics in the food.

It is well know that when infection occurs, fish do not take the medicated feed, and they become anorexic immediately post-infection; thus, bath treatment seems to be more effective than the oral treatment (Soltani et al., 1996). In cases where the infection is located on the skin surface, bath treatment with potassium permanganate or quaternary ammonium can be used (Bernardet, 1998; Noga 2000). According to Avendaño-Herrera et al. (2008) bath treatment with enrofloxacin 30 ppm, for 1h and for 3 days, is also very effective.

An alternative method in the use of antibiotics is the use of antiseptic solutions with bath treatment, like formalin and iodide solutions. Bath treatments with formalin (30-40 ppm for 6h) proved to be effective in the control of tenacibaculosis in sole, *Dover sole* (McVicar and White, 1979). However, lesions in gills were found and connected with the prolonged use of formalin. Iodide solutions have been used mainly for the disinfection of fish eggs and equipment of aquaculture farms (Hirazawa et al., 1999; Cipriano et al., 2001; Costello et al., 2001; Tendencia, 2001).

In 2012, Mohamed et al., proved with in vitro experiments in fish that the addition of carvacrol (main ingredient of oregano with antibacterial properties), in a concentration 100 ppm in food for 14 days, is effective in the control of the disease, reducing mortality. Temperature and/or salinity control, can also been used to reduce symptoms from *T. maritimun* in salmons. Soltani and Bucker (1994), proposed to maintain the temperature at 15^{o}C and salinity under 10g l^{-1}.

Hydrogen peroxide (H_2O_2) has recently received considerable attention for its effective use of numerous external pathogens to fish. Hydrogen peroxide, 35% (PEROX-AID®) has been used in aquaculture with bath treatment for external parasites, bacteria and fungus in different species and ages of fish (Kiemer and Black, 1997; Treasurer and Grant, 1997; McAndrew et al., 1998; Treasurer et al., 2000; Grant, 2002). Moreover, hydrogen peroxide is friendly to the water environment, as it decomposes into water and oxygen (Kiemer and Black, 1997).

Avendaño-Herrera et al. (2006a), examined whether this chemical disinfectant at concentrations ranging from 30 to 240 ppm has the capacity to kill *T. maritimun* in in vitro assays, in turbot. Based on the results, they recommend the use of hydrogen peroxide at a concentration of 240 ppm as a general disinfection preventive method for treating water culture and surface of tanks before the introduction of fish.

Chloramine-T, (Halamid®) is considered a treatment choice in salmons, rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), Atlantic sturgeon (*Acipenser oxyrhynchus*) and other fish species that are affected from Bacterial gill disease (BGD) or flexibacteriosis of freshwater fish. Experiments in vitro in fish have proved that bath treatment with 10-20 mg l^{-1}chloramine-T for 60min, 3 baths every other day, is very effective for the prevention or control of external parasites and bacteria (Powell and Perry 1997; Harris et al., 2004, 2005; Leef et al. 2007; Boran and Altinok, 2013).

Recently, experimental infections and immersions with hydrogen peroxide and chloramine-T, in experimental infected sea bream and sea bass were performed, in order to control their effectiveness in the treatment of tenacibaculosis. After the completion of the experiments with the two solutions, fish responded to the therapy, reducing the mortality rate. The immersions were made with: hydrogen peroxide (solution 35%), in the dose 200 ppm for 30min and chloramine-T, in the dose 12-15ppm for 60min, suggesting that these 2 antiseptic solutions could be used in the future for the effective treatment of the disease (Gourzioti, 2014).

PREVENTION

Until recently, no vaccines were available to prevent the disease, but a vaccine (FM 95) has been patented by the University of Santiago (Spain) and is the only bacterin currently in the market to prevent mortalities caused by *T. maritimun* in turbot (Santos

et al., 1999). Because this disease affects juvenile and adult turbot, the vaccine is applied by bath when the fish are 1–2 g and later by injection when the fish attain 20–30 g. The percentage of protection by bathing is about 50%, but when the vaccine is administered by i.p. injection the protection increases to more than 85%. Polyvalent formulations to prevent tenacibaculosis and vibriosis or tenacibaculosis and streptococcosis in turbot are also available (Toranzo et al., 2005).

Unfortunately, because of the high degree of serological diversity of *T. maritimun*, the vaccine is not reported effective in other species of fish, except turbot. Therefore, a new tenacibaculosis bacterin specific for cultured sole is currently being developed by Romalde et al., and this has conferred RPS values higher than 90% in laboratory trials performed by i.p. injection (Romalde et al., 2005).

CONCLUDING REMARKS

Tenacibaculosis has been reported in many aquaculture farmed marine fish worldwide, causing significant losses of fish, but there are no extensive studies for the disease in Mediterranean fish species. Also, isolation of the bacterium *T. maritimun*, remains difficult because requires specialized knowledge and specific substrates. Cultivation and identification of the most pathogenic strains could help in finding more specific and rapid techniques for faster and effective treatment. Despite the studies until now, there is a lack of knowledge concerning non specific defense factors, virulence factors, the survival and transmission of the pathogen.

In the future, further investigations are required for the valid identification of the bacterium, for determining the sources of contamination, mechanisms of transmission and virulence, for the use of new choices of prevention and treatment (hydrogen peroxide, chloramine-T) and the development of new vaccines against the disease. It is also necessary, a thorough research on the pathology of new fish species entering aquaculture farms, like *Umbrina Cirrosa* and *Sciaena umbra*, in order to facilitate their farming in aquaculture.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

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