

Tenacibaculosis in marine fish

Tenacibaculum infection (previously named flexibacteriosis) represents one of the first bacterial conditions being reported in marine aquaculture in a large range of species including flat fish such as sole and turbot; and finfish, namely sea bass and sea bream (McVicar and White, 1979; Masumura & Wakabayashi, 1977; Bernardet, 1989; Toranzo et al., 2005). More recently, at the 2018 Annual Workshop of the National Reference Laboratories for Fish Diseases (Anonymous, 2018) or from the Assessment of the disease situation made by MedAID (Cidad et al., 2018) tenacibaculosis was considered, together with vibriosis, one of the most important disease for European seabass. In some marine areas or in recirculation systems, they severely threaten production with mortalities rising over 30%.

Causative agent

Bacteria from the genus *Tenacibaculum* are Gram-negative filamentous bacteria, 2-30 µm long and 0.5 µm in diameter, show gliding motility on wet surfaces, and produce catalase and cytochrome oxidase (Wakabayashi et al., 1986). Colonies are flat, pale yellow with irregular margins and adhere strongly to FMM agar, absorb Congo red but do not contain a cell-wall-associated flexirubin-type pigment (Avendano-Herera et al., 2004). All tested strains of *T. maritimum* are biochemically homogeneous but antigenic heterogeneity was established due to differences in the "O" chains of LPS revealing 3 major O-serotypes from strictly marine fishes which are host specific; O1 is predominant in sea bream and sea bass, O2 in turbot, O1 and O3 but also O2 in sole, and O3 and recently also O2 in salmon. Pathological properties of the bacterium, such as a strong adherence to the skin mucus of different fish species and the capacity to resist its bactericidal activity have been pointed out as possible virulence factors (Margarinos et al., 1995). Pazos (1997) proved that extracellular products (ECP) of this species have very high proteolytic activity with an ability to degrade gelatin, amylase, casein and nucleases.

Epidemiology of the disease

Pathological changes caused by tenacibaculosis are generally affecting fish from nursery phase to pre-growing fish up to 80-100 grams average weight. Prevalence and intensity of the infections vary in relation to environmental parameters and zootechnical rearing conditions (Avendano-Herera et al., 2006). Severity of clinical symptoms and mortality rates are influenced by fish size, water quality parameters and temperature, rearing factors such as handling, mechanical lesions or stress and interfering parasitic infestations (Brevik, 2015). An increased prevalence and severity of the disease has been reported at higher temperatures, above 15°C (Toranzo et al., 2005).

There is no host specificity, and wild fish may serve as reservoirs of infection. Some studies allude the involvement of jellyfish (Ferguson et al. 2010) and sea lice (Barker et al. 2009) as vectors of the bacterium. Chronic presence of the bacterium in the mucus layer suggests fish mucus to be a reservoir (Avendano-Herrera et al., 2006). Many studies support the hypothesis on *Tenacibaculum* spp. as an opportunistic pathogen that primarily causes extensive skin lesions and gill abrasion, and subsequent systemic infections (Avendano-Herrera et al., 2006). Wakabayashi et al. (1986) proved that topical application of bacterial culture on the mouth or tail causes a fatal infection, while intraperitoneal and artificial intramuscular infection does not cause disease (Avendano-Herrera et al., 2006).

Clinical signs

Clinical signs of tenacibaculosis are variable according to fish species and type of infection. Affected fish show loss of appetite, become lethargic and show skin lesions around the eyes, on the head, and also bone nodules are seen. The lesions are characterized by increased mucus production and presence of whitish necrotic tissue (Smage et al., 2016). Characteristic lesions on the body surface of fish are described, such as ulcers, necrosis, eroded mouth, frayed fins and tail rot, and sometimes necrosis of the gills and eyes (McVicar and White, 1979; Campbell and Buswell, 1982; Wakabayashi et al. 1986; Baxa et al., 1986, Devesa et al. 1989; Chen et al. 1995, Handlinger et al., 1997; Ostland et al., 1999; Cepeda & Santos, 2002). Fish with gill infections may display an increased respiratory rate (Mitchell and Rodger, 2011) with visible yellow or brown mats on the pale gills, and extensive areas of severe necrosis. Although all age classes may be infected, younger fish suffer a more severe form of the disease. The onset of disease is influenced by a multiplicity of environmental and host related factors, such as skin surface condition (Margarinos et al., 1995). A systemic disease may also result involving different internal organs. The loss of epithelial fish surface, typical of this disease, is also a port of entry for other bacterial or parasitic pathogens. In the Mediterranean area those infections are initially external, affecting the dermal and epidermal layers. Lesions usually develop as co-infections, becoming ulcerative and tenacibaculosis very often affects sea bass in a mixed infection with *V. anguillarum* at the end of the winter, causing high and persistent mortalities (Zrnčić et al. 2013).

Diagnostics

Diagnosis is done on fresh smears from scrapping of the lesions by light microscopic observation directly or after quick staining (MGG/RAL555). *Tenacibaculum maritimum* is observed as long, gliding Gram negative bacteria (Le Breton, *personal communication*).

For the past five years, clinical symptoms observed are more diversified, including deeper ulcerative lesions. Correlatively, increasing failure in treatments are reported. Monitoring and research programs such as the FUI Pathotrackerfish have confirmed that *T. maritimum* actually represents only 50% of the strains isolated from clinical cases (Arnaud et al., 2018). Diagnostic methods recently applied to fish pathogen identification based on proteomic fingerprinting using high-throughput mass spectrometry MALDI-TOF BRUCKER spectrometry (Toranzo, 2015) or genomics (MLST, WGS) confirm the presence or the emergence of different pathogenic species (Habib et al., 2014). In most cases, co-infections by at least two *Tenacibaculum* strains are noticed, which may explain the evolution of clinical aspects, different pathogenicity patterns and poor treatment efficacy.

Disease management

Disease outbreaks through *T. maritimum* can be avoided or reduced by controlling fish density, reducing stress conditions, avoiding overfeeding as well as manipulating the water temperature (15°C), and lowering the salinity to 10g/l (Soltani et al., 1996).

The accurate diagnostic might open the possibility for development of the efficient vaccine. Currently there is a single commercial vaccine for turbot in Spain (Romalde et al., 2005), and several vaccines against tencibaculosis were tested for different marine fish species as for barramundi in Singapore (Rafidah et al., 2015), salmon in Tasmania (Van Gelderen et al., 2009) or salmon in Chile (Tobar, 2015).

However, the accurate identification of *Tenacibaculum* strains from sea bass and sea bream opens the field for preparation of monovalent or polyvalent auto-vaccines adapted to each situation.

In vitro susceptibility studies of isolates from different species and different geographical regions exhibit a similar pattern, with susceptibility to tetracyclines, potentiated sulphonamides and fluoroquinolones, and a resistance to kanamycin, neomycin, and quinolones (Avendano-Herrera et al. 2006). However, very often field results are not coherent with *in vitro* studies. So far as infected fish become anorexic very soon after infection, bath treatment seems to be more effective than oral (Soltani et al. 1995).

The use of specific media for cultivation and *in vitro* antibiotic sensitivity testing, as well as swabs with adapted transport media, allow a better isolation and identification of these strains. This is a key point to apply efficient therapeutic treatments. Still, many questions remain without answers in the field.

References :

- Anonymous (2018). Report from the 22nd annual workshop of the National Reference Laboratories for Fish Diseases. EURL. (<http://www.mediterranean2020.eu/wp-content/uploads/2018/07/22nd-AW-report-2018.pdf>).
- Arnaud, M., P.Y. Moalic, F. Bourgeois, R. Carpentier, A. Le Breton. 2018. *Tenacibaculum* infections in marine finfish aquaculture: recent evolution in clinical aspects and strain identification – impact on prophylactic methods. AQUA 2018, Meeting of the World Aquaculture Society and the European Aquaculture Society. 25 – 29 August, Montpellier, France.
- Avendano-Herrera, R., A.E. Toranzo, B. Magarinos. 2006. *Tenacibaculum* infection in marine fish caused by *Tenacibaculum maritimum*: a review. *Diseases of Aquatic Organisms*, 71: 255-266
- Barker, D.E., L.M. Braden, M.P. Coombs, B. Boyce. 2009. Preliminary studies on the isolation of bacteria from sea lice, *Lepeophtheirus salmonis*, infecting farmed salmon in British Columbia, Canada. *Parasitology Research*, 105: 1173–1177.
- Baxa, D.V., K. Kawai, R. Kusuda. 1986. Characteristics of gliding bacteria isolated from diseased cultured flounder, *Paralichthys olivaceous*. *Fish Pathology*, 21: 251–258
- Bernardet, J.F. 1998. *Cytophaga*, *Flavobacterium*, *Flexibacter* and *Chryseobacterium* infections in cultured marine fish. *Fish Pathology*, 33: 229-238
- Brevik, Ø. 2015. The impact of *Tenacibaculum* in Norway. In *Tenacibaculum maritimum*: Current Knowledge and Future Directions. Workshop Report and Synopsis, Campbell River, Canada, 15-16 Feb.
- Campbell, A.C., J.A. Bushwell. 1982. An investigation into bacterial aetiology of « black patch necrosis » in Dover sole, *Solea solea*. *Journal of Fish Diseases*, 5 : 495-508
- Cepeda, C., Y. Santos. 2002. First isolation of *Flexibacter maritimus* from farmed Senegalese sole (*Solea senegalensis*, Kaup) in Spain. *Bulletin of the European Association of Fish Pathologists*, 22: 388–392
- Chen, M.F., D. Henry-Ford, J.M. Groff. 1995. Isolation and characterization of *Flexibacter maritimus* from marine fishes of California. *Journal of Aquatic Animal Health*, 7: 318–326
- Cidad, M., Peral, I., Ramos, S., Basurco, B., López-Francos, A., Muniesa, A., Cavallo, M., Pérez, J., Aguilera, C., Furones, D., Reverté, C., Sanjuan-Vilaplana, A., Brun, E., Jansen, M.D., Tavoranpanich, S., Raux, P., Baraibar, E., Cobo, A., Fernández-Polanco, J.M., Llorente, I., Fernández Sánchez, J.L., Luna, M., Luna, L., Odriozola, M., Gulzari, B., Janssen, K., Komen, H. (2018). Assessment of Mediterranean Aquaculture Sustainability. Deliverable 1.2 of the Horizon 2020 project MedAID (GA number 727315), published in the project web site on 21.12.2018 : <http://www.mediterranean2020.eu/index.php/deliverables/>
- Devesa, S., J.L. Barja, A.E. Toranzo. 1989. Ulcerative skin and fin lesions in reared turbot, *Scophthalmus maximus* (L). *Journal of Fish Diseases*, 12: 323–333

Ferguson, H.W., C.M.J.Delannoy, S.Hay, J.Nicolson, D.Sutherland, M.Crumlish. 2010. Jellyfish as vectors of bacterial disease for farmed salmon (*Salmo salar*). *Journal of Veterinary Diagnostic Investigation*, 22: 376–382.

Habib, C., A. Houel, A. Lunazzi, J-F. Bernardet, A.B. Olsen, H. Nilsen, A.E. Toranzo, N. Castro, P. Nicolas, E. Duchauda. 2014. Multilocus Sequence Analysis of the Marine Bacterial Genus *Tenacibaculum* Suggests Parallel Evolution of Fish Pathogenicity and Endemic Colonization of Aquaculture. *Applied and Environmental Microbiology*, 80: 5503–5514.

Handlinger, J., M. Soltani, S. Percival. 1997. The pathology of *Flexibacter maritimus* in aquaculture species in Tasmania, Australia. *Journal of Fish Diseases*, 20: 159–168

Magarinos, B., F. Pazos, Y. Santos, J. L. Romalde, A. E. Toranzo. 1995. Response of *Pasteurella piscicida* and *Flexibacter maritimus* to skin mucus of marine fish. *Diseases of Aquatic Organisms*, 21: 103–108.

Masumura, K., H. Wakabayashi. 1977. An outbreak of gliding bacterial disease in hatchery-born red seabream (*Pagrus major*) and gilthead (*Acanthopagrus schlegelii*) fry in Hiroshima. *Fish Pathology*, 12: 171–177

McVicar, A.H., P.G. White. 1979. Fin and skin necrosis of cultivated Dover sole *Solea solea* (L). *Journal of Fish Diseases*, 2: 557–562

Mitchell, S.O., H.D. Rodger. 2011. A review of infectious gill disease in marine salmonid fish. *Journal of Fish Diseases*, 34: 411–432.

Ostland, V.E., C. LaTrace, D. Morrison, H.W. Ferguson. 1999. *Flexibacter maritimus* associated with a bacterial stomatitis in Atlantic salmon smolts reared in net-pen in British Columbia. *Journal of Aquatic Animal Health*, 11: 35–44

Småge, S.B., K. Frisch, O. Jakobsen Brevik, K. Watanabe, A. Nylund. 2016. First isolation, identification and characterisation of *Tenacibaculum maritimum* in Norway, isolated from diseased farmed sea lice cleaner fish *Cyclopterus lumpus* L. *Aquaculture*, 464, 178–184

Soltani, M., B.L. Munday, C.M. Burke. 1996. The relative susceptibility of fish to infections by *Flexibacter columnaris* and *Flexibacter maritimus*. *Aquaculture*, 140: 259–264

Toranzo A.E., B. Magarinos, J.L. Romalde. 2005. A review of the main bacterial fish diseases in mariculture systems. *Aquaculture*, 246: 37–61

Toranzo, A.E. 2015. Tenacibaculosis of farmed fish in Southern Europe. In *Tenacibaculum maritimum: Current Knowledge and Future Directions*. Workshop Report and Synopsis, Campbell River, Canada, 15–16 Feb.

Van Gelderen, R., J. Carson, B. Nowak. 2009. Experimental vaccination of Atlantic salmon (*Salmo salar* L.) against marine flexibacteriosis. *Aquaculture*, 288: 7–13.

Zrnčić, S., D. Oraić, I. Račić, R. Beck. 2013. *Tenacibaculum maritimum* isolated from sea bass (*Dicentrarchus labrax*) in Croatia. 16th International Conference on Diseases of Fish and Shellfish. Tampere, Sept. 2–6, 2013, p. 387.