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Στρεπτοκοκκικές λοιμώξεις στα εκτρεφόμενα ψάρια

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Streptococcal infections of farmed fish

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ΠΕΡΙΛΗΨΗ. Το γένος *Streptococcus* περιέχει ένα μεγάλο αριθμό Gram θετικών βακτηρίων. Μόνον μερικοί βιότυποι έχουν απομονωθεί στα ψάρια και τα πιο παθογόνα είδη ανήκουν στην ομάδα με ορότυπο D που είναι γνωστά ως Enterococci. Σηψαιμία από τα βακτήρια αυτά για πρώτη φορά αναφέρθηκε στην ιριδίζουσα πέστροφα (*Onchorynchus mykiss*) στην Ιαπωνία το 1958. Από τότε η ασθένεια εμφανίζεται σποραδικά σε εκτρεφόμενα ή άγρια ψάρια τόσο γλυκού όσο και θαλασσινού νερού σε όλον τον κόσμο. Τα είδη *Enterococcus* sp. προκαλούν σημαντικές οικονομικές επιπτώσεις, ειδικά στα θαλάσσια είδη. Από τα ψάρια γλυκού νερού η πέστροφα (*Onchorynchus mykiss*) και η Τιλάπια (*Oreochromis niloticus*) είναι τα πιο σημαντικά είδη ψαριών που προσβάλλονται. Τα κλινικά συμπτώματα διαφέρουν αναλόγως με τα προσβεβλημένα είδη ψαριών. Τα πιο συχνά συμπτώματα είναι ανορεξία, ανωμαλίες κολύμβησης, σκούρος χρωματισμός, οφθαλμικές αλλοιώσεις, εξωτερικές αιμορραγικές αλλοιώσεις και εξελκώσεις. Το σύστημα ταυτοποίησης βασίζεται στην ανταγωνικότητα του κυτταρικού τοιχώματος και γίνεται με βάση το σύστημα του Lancefield, όπου τα βακτήρια διακρίνονται σε *Enterococcus* ή *Enterococcus*-like streptococci με βάση χαρακτηριστικά, όπως η αιμόλυση και τα βιοχημικά χαρακτηριστικά. Με βάση αυτά τα κριτήρια μερικά στελέχη έχουν χαρακτηριστεί ως ταυτόσημα. Έχει βρεθεί ότι τα στελέχη από ψάρια γλυκού νερού στην Αμερική ανήκουν στην ομάδα B (Lancefield's group B), ενώ ερευνητές στην Ιαπωνία, αφού ανέλυσαν πολυάριθμα στελέχη από ψάρια του είδους *Seriola* sp. από θαλασοκαλλιέργειες, αλλά και από χέλια, με βάση τη μέθοδο του υβριδισμού κατέληξαν στο συμπέρασμα ότι τα βακτήρια είναι πιο κοντά στο γένος *Enterococcus* και ότι η νόσος στην Ιαπωνία στα θαλασσινά ψάρια θα πρέπει να ονομάζεται "Enterococcal infection". Δεξαμενή για τη μετάδοση της νόσου είναι τα βακτήρια που αιωρούνται από τα ασθενή ψάρια. Τα βακτήρια αυτά παραμένουν στην ιλύ γύρω από τις εκτροφές για μεγάλο χρονικό διάστημα. Μεγαλύτερος αριθμός βακτηρίων υπάρχει στο θαλασσινό νερό το καλοκαίρι, ενώ τους χειμερινούς μήνες αυτός είναι μεγαλύτερος στην ιλύ του πυθμένα. (Kिताo et al. 1979). Σε ψάρια γλυκού νερού έχει βρεθεί ότι η νόσος έχει σχέση με το οργανικό φορτίο και τη μόλυνση σε ποταμούς που τροφοδοτούν

Streptococcal infections of farmed fish

Athanassopoulou F*¹, Roberts R.J.²

ABSTRACT. The genus *Streptococcus* is large and complex, accommodating a wide range of Gram positive bacteria. Only a few biotypes have been isolated from fish and the most pathogenic are those belonging to D serogroup, otherwise known as the Enterococci. Streptococcal septicaemia was first among cultured rainbow trout (*Onchorynchus mykiss*) in Japan in 1958. Since then, the disease has appeared sporadically or in epizootics among cultured or wild fish both in freshwater and marine environments all over the world. Among the freshwater species infected, rainbow trout (*Onchorynchus mykiss*) and tilapia (*Oreochromis niloticus*) are the most important species. Clinical signs vary among species of affected fish. The most common symptoms are loss of appetite, erratic swimming, darkening of body colour, eye lesions, external haemorrhagic lesions and ulcerations. The standard system for identification is based on the antigenicity of the carbohydrate moiety of the cell wall, according to a scheme devised by Lancefield in the 1930's. The American freshwater isolates obtained by Robinson & Meyer (1966), Plumb et al. (1974), Rasheed & Plumb (1984) were all typed Lancefield's group B serotype in contrast to almost all of the much more frequent isolates from marine fish, which are usually untypable *Enterococcus*-like strains. The *Streptococcus* strains isolated from yellowtail (*Seriola* sp), ayu (*Plecoglossus altivelis*) and flounder (*Rhombosolea* sp) have similar biochemical reactions to *Streptococcus iniae*, a species isolated from dolphins by Pier & Madin (1976), which is defined in Bergey's Manual as a separate species. Kusuda et al. (1991) have analysed numerous strains from yellowtail infections in mariculture and in eels and have concluded, on the basis of DNA/DNA hybridisation studies and biochemistry that a specific condition, caused by such streptococci, exists and that these bacteria were closest to Enterococci in characteristics and that infectious coxosis of marine fish, as seen in Japan, should be called "Enterococcal infection". Enterococci released from diseased fish seem to be the main source of infection. It has been shown that these bacteria remain in seawater and particularly in the mud around farms for a long time. Higher numbers of microorganisms exist in the seawater during summer months in contrast to the winter months when the bacterial

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εκτροφές και ειδικά σε θερμοκρασίες 21-22°C (Ghittino & Praero 1992). Τα άγρια ψάρια μπορούν, επίσης, να μεταδώσουν τη νόσο. Πιστεύεται ότι η μετάδοση είναι άμεση από μολυσμένα ψάρια και τροφή. Οι μηχανισμοί της παθογένειας της νόσου δεν είναι πλήρως κατανοητοί.

Λέξεις ευρετηρίασης: *Streptococcus*, *Enterococcus* infections, cultured fish.

1. INTRODUCTION

The genus *Streptococcus* is large and complex, accommodating a wide range of Gram positive bacteria. They obtain their name from the characteristic division along an horizontal axis in liquid media to produce chains of round cells (streptos=chain, coccus=globe). They are commensals of humans and animals. Only a few biotypes have been isolated from fish and the most pathogenic are those belonging to D serogroup, otherwise known as the Enterococci. Enterococci are part of the enteric microflora and are indicators of faecal contamination of the aquatic environment. Also they are recognized as pathogens for both higher animals and fish.

Streptococcal septicemia was first reported among cultured rainbow trout (*Onchorynchus mykiss*) in Japan in 1957 (Hoshina et al. 1958). Robinson & Meyer (1966) reported two epizootics in golden shiner (*Notemigonus crysoleucas*) and the bacterium was isolated from diseased fish involved in epizootics in estuarine bays along the Alabama and Florida coast of Gulf of Mexico (Plumb et al. 1974). Since then, the disease has appeared sporadically or in epizootics among cultured or wild fish both in freshwater and marine environments all over the world.

2. Species specificity and distribution

Enterococcosis of cultured fish has been described in a wide range of species: among the freshwater species infected, rainbow trout (*Onchorynchus mykiss*) and tilapia (*Oreochromis niloticus*) are the most important species affected. The bacterial species involved were *Streptococcus shiloi* and *Streptococcus difficile* causing meningoencephalitis in fish in Israel (Eldar et al. 1994). *Streptococcus faecalis* was also reported from rainbow trout from northern Italy (Ghittino & Praero 1992), whereas Eldar et al. (1996) isolated *Enterococcus seriolicida* from diseased rainbow trout from Italy also, suffering from septicemia and meningoencephalitis. *Enterococcus* sp. causes economically important losses in marine culture fish. Apart from Japan, outbreaks have been reported from cultured marine fish in Singapore (Foo et al. 1985), USA (Plumb et al. 1974; Baya et al. 1990; Chang & Plumb 1996), Spain (Toranzo et al. 1996), Greece (Varvarigos 1998) and

load is higher in the mud (Kitao et al. 1979). Ghittino & Praero (1992) have shown that in freshwater fish the outbreak of the disease was related to an organic pollution of the rivers supplying fish farms, associated with high water temperature (21-22°C). Wild fish can also be infected and spread the bacteria. It is generally believed that the infection is horizontal with infection occurring from direct contact with infected fish or contaminated fish food (Robinson & Meyer, 1996). The mechanisms of enterococcal infections pathogenicity are not yet fully understood.

Key words: *Streptococcus*, *Enterococcus* infections, cultured fish.

Kuwait (Evans et al. 2002). It is only in Japan, however, that it has been recognized as a very serious and economically important disease of cultured fish (Hoshina et al. 1958; Kusuda et al. 1976; Kitao 1982; Aoki et al. 1990; Kusuda et al. 1991) and it has developed as the important disease in yellowtail aquaculture industry (Alim et al. 1996). The condition is not only confined to yellowtail (*Seriola* sp) and sea farmed rainbow trout. Ayu (*Plecoglossus altivelis*), salmonids (*Onchorynchus* spp), eels (*Anguilla anguilla*), flounder (*Rhombosolea* sp) and amberjack (*Pleuronectes flesus*) have also been found to develop the condition involving infection with the bacterial species now designated as *Streptococcus seriolicida*, (after *seriola*=yellowtail its principal host in Japanese waters) (Minami 1979; Iida et al. 1982, 1986; Kawahara & Kusuda 1987; Kusuda et al. 1991; Kitao 1993).

In Singapore, mass mortality was reported in rabbitfish (*Siganus canaliculatus*) (Foo et al 1985) as a result of *Enterococcus* and in the USA, whereas Baya et al. (1990) described a serious outbreak in Chesapeake Bay in wild fish involving blue fish, striped bass and sea trout. In Spain, isolated outbreaks have been reported in coastal turbot culture (Toranzo et al. 1995, 1996) involving two strains *Streptococcus parauberis* and an *Enterococcus*-like bacterium which resembles *Enterococcus seriolicida* (Toranzo et al. 1995, 1996 Domenech et al. 1996). In Canada, human disease was associated with handling of farmed Atlantic salmon (Weinstein et al. 1997) and strains from fish were also isolated (Ferguson et al. 1994). The disease has also been reported from Australia, South Africa (Carson et al. 1993) and Saudi Arabia (Alharbi 1994). *Streptococcus iniae* was isolated from diseased wild fish, collected near a mariculture facility, where gilthead sea bream and European sea bass exhibited a similar infection. Species-specific PCR and ribotyping confirmed that wild and cultured fish were infected by a single *S. iniae* clone (Zlotkin et al. 1998). The condition has been of interest also in Greece, the leading producer of sea bream *Sparus aurata* L. and bass *Dicentrarchus labrax* L. in the Mediterranean Sea. Sporadic reports and information exist that the disease has occurred throughout the Greek mariculture industry at increasing levels since 1995. Fish involved are principally sea bream and bass (0.5-3g of weight), but also newly

Πίνακας 1. Διάγνωση στρεπτοκόκκων**Table 1.** Presumptive identification of streptococci

Category	Cellular arrangement	Haemolysis	Group antigen	SXT resistance	BE test	Salt tolerance
Group A strept.	chains	beta	A	yes	--	--
Group B strept.	chains	beta	B	yes	--	+
"Groupable" beta-haemolytic streptococci, not group A, B, or D	chains	beta	Not A, B, or D	no	--	--
Group D, enterococci	short chains, diplo	alpha, beta, none	D	yes	+	+
Group D, not enterococci	short chains, diplo	alpha, none	D	no	+	--
Viridans streptococci	chains	alpha, none	none	no	--	--

established fish species, such as *Dentex dentex* and *Puntazzo puntazzo*, are also affected (Varvarigos 1998; Athanassopoulou et al. 1999; Prapas, pers. com.). There is no detailed data on these species, but field evidence suggests (Prapas, unpublished data) that the condition is an acute bacterial septicaemia characterized by the presence of Staphylococci, Streptococci and Enterococci. In some cases, mixed infections with Gram negative bacteria, such as *Pasteurella* and *Vibrio* sp., are also present, especially in later stages of the development of the disease.

3. Clinical signs and pathology

Clinical signs vary among species of affected fish. The most common symptoms are loss of appetite, erratic swimming, darkening of body colour, eye lesions, external haemorrhagic lesions and ulcerations (Inglis et al. 1993). The haemorrhagic lesions are raised with a dark zone around them; these are more superficial than the furunculosis or *Vibrio* lesions, but gradually they ulcerate (Chang & Plumb 1996).

Areas particularly affected are the dorsum, the area anterior to the caudal peduncle, the opercula, around the mouth, the anal fin and the vent. (Roberts 1987).

Histologically, the lesions are characteristic in the eye, where exophthalmus due to retrobulbar oedema and congestion is followed by cellular infiltration into the choroids and necrosis of orbital elements. Ultimately, haemorrhage into the eye may lead to evulsion of the entire orbital content (Roberts 1987). Other findings comprise hyperaemia of bronchial vessels, macrophage infiltration of gill lamellae and rupture of secondary lamellae leading to acute mortality. The internal organs affected include mainly the spleen and the liver, but also the kidney and the heart are affected by infarctive local haemorrhage and necrosis, which characterize the condition. Pericarditis,

fibrohaemorrhagic adhesions of pericardium and of visceral and parietal peritoneum are also findings occurring frequently (Plumb et al. 1974; Kusuda et al. 1976; Kitao et al. 1981; Jo 1982; Baya et al. 1990; Kitao 1993; Robinson & Meyer 1966). Nieto et al. (1995) have described two forms of the disease in turbot, a focal form involving eye, skin lesions and meningitis and a generalized form affecting most internal organs with purulent exudate in the peritoneal cavity. Often in streptococcal infections, it is extremely difficult to find the bacteria even in tissue sections stained with specialized stains possibly due to the fact that the lesions have been caused by the exotoxins released from a specific focus of bacteria elsewhere (Sanjeev & Surendran 1992).

4. Aetiological Agent

The genus *Streptococcus* includes a wide range of Gram-positive bacteria which are spherical or oval and generally less than 2 µm in diameter forming pairs or chains when grown in liquid media. They are generally non-motile, facultative anaerobic and chemo-organotrophic. Their metabolism is fermentative and have a G+C ratio of DNA between 34-46% (Inglis et al. 1993).

The standard system for identification is based on the antigenicity of the carbohydrate moiety of the cell wall, according to a scheme devised by Lancefield in the 1930's (Lancefield 1933); (Lancefield typing criteria have been colloquially referred to *Enterococcus* or *Enterococcus*-like streptococci based on other characteristics, such as haemolysis and biochemical features. Recently, based on these features, some strains have been renamed with synonyms (Eldar et al. 1995a, 1996).

5. Cultural and biochemical characteristics

There is considerable variation in biochemical

Πίνακας 2. Χαρακτηριστικά των β-αιμολυτικών και μη αιμολυτικών στρεπτοκόκκων του group B από διάφορα είδη ψαριών
Table 2. Characteristics of β-haemolytic group B and non-haemolytic group B *Streptococcus* from different fish species

Test	β-haemolytic		Non-haemolytic		
	Sakai et al. (1936)	Eldar et al. (1994)	Baya et al. (1990)	Wilkinson, Thacker & Facklam (1973)	Robinson & Meyer (1966)
OF test	-/-	+/+	-/-	-/-	nr
Haemolysis	β	Non-haemolytic	Non-haemolytic	Non-haemolytic	Non-haemolytic
Catalase	-	-	+	-	nr
Oxidase	-	-	-	-	nr
Acetoin production	+	+	+	nr	nr
Hippurate	+	-	nr	+	nr
Esculin	-	-	nr	nr	nr
Starch	-	-	-	-	-
Pyrrolidonylarylamidase	-	-	nr	nr	nr
α-Galactosidase	-	-	nr	nr	nr
β-Glucorinase	-	-	nr	nr	nr
β-Galactosidase	-	-	nr	nr	nr
Alkaline phosphatase	+	+	nr	nr	nr
Leucine arylamidase	+	+	nr	nr	nr
Arginine dihydrolase	+	+	+	nr	nr
Mannitol	-	-	-	nr	nr
Growth at					
10°C	-	-	(15°C)	nr	nr
37°C	+	-	+(30°C)	nr	nr
45°C	-	-	(40°C)		nr
Growth at pH 9.6	-	+	nr	nr	nr
Growth in media containing					
4.0% NaCl	+	nr	nr	+	-
6.5% NaCl	-	-	(6.0%)	-	-
10% bile	-	nr	nr	-	-
40% bile	-	-	nr	-	-

OF test, oxidation, fermentation test; nr, not reported.

properties between isolates. The isolation of these bacteria is greatly enhanced by the use of blood based media, which allow demonstration of the haemolytic property which is sometimes used to subdivide the group. Sakai et al. (1993b) suggested that the API ZYM rapid system of identification, with additional tests, such as Gram stain and cytochrome oxidase and catalase, are sufficient to distinguish several biotypes (Tables 1 & 2).

6. Serology and antigenic variations

Different serotypes of *Streptococci* can be tested by precipitations ring tests, by gel diffusion (Rotta et al. 1971) or counter current electrophoresis (Dajani, 1973). In each case an extract of the group specific antigens is used, which can be obtained by hot acid extraction, hot formamide (Fuller 1938) or the autoclave extraction of Rantz &

Randall (1995). Other techniques include coagglutination (Christensen et al. 1973) and immunofluorescence (Cars et al. 1975). Sera for typing are normally obtained in standardized series from commercial diagnostic reagent suppliers. The American freshwater isolates obtained by Robinson & Meyer (1966), Plumb et al. (1974), Rasheed & Plumb (1984) were all typed as falling into Lancefield's group B serotype in contrast to almost all of the much more frequent isolates from marine fish, which are usually untypable *Enterococcus*-like strains. The *Streptococcus* strains isolated from yellowtail (Minami et al. 1979), from ayu (Ohnishi & Jo, 1981), from flounder (Nakatsugawa 1983) and from yellowtail by Kaige et al. (1984), all have similar biochemical reactions to *Streptococcus iniae*, a species isolated from dolphins by Pier & Madin (1976), which is defined in Bergey's Manual (Hardie 1986) as a separate species. It is not able to be classified by

Lancefield's scheme. Kawahara & Kusuda (1987) have subsequently shown by immunofluorescence that these strains all serologically cross react with *Streptococcus iniae*. The exact taxonomic position of *S. iniae* from fish within the serological scheme which is the basis of classification, is not therefore, certain. Some strains from similar outbreaks in yellowtails in Japan differed from *S. iniae* strains in being a haemolytic. They had characteristics of *Streptococcus faecalis* and *Streptococcus faecium* (Kusuda et al. 1976; Kitao 1982, 1983), but again they did not react with Lancefield Group D antisera. Kusuda et al. (1991) have analysed numerous strains from yellowtail infections in mariculture and in eels and have concluded, on the basis of DNA/DNA hybridisation studies and biochemistry, that a specific condition caused by such streptococci exists and that these bacteria were closest to Enterococci. Therefore, this infection of marine fish, as seen in Japan, should be called "Enterococcal infection" (Kusuda et al. 1991). Toranzo et al. (1995) tested polyclonal antisera against strains of virulent *Enterococcus* strains in turbot and found that serological identification of *Enterococcus* strains in turbot, using these methods, were very problematic due to cross reactions with other group of bacteria. In contrast to this, Leiro et al. (1996) found that specific turbot serum antibodies, raised against isolate of an *Enterococcus* strain pathogenic to turbot, did not cross react in enzyme-linked immunosorbent (ELISA) with other enterococcal or non-enterococcal Gram positive bacteria.

7. Diagnosis

Presumptive diagnosis can be made by the typical clinical signs and the isolation of Gram positive bacteria from the brain and internal organs of affected fish. Bacteria are best isolated from the brain (Sugiyama et al. 1981). Todd Hewitt medium, brain heart infusion or heart infusion media containing 0.5% glucose at 25-30°C are normally used for the isolation of the bacteria in the laboratory. A definite diagnosis requires serological typing and fluorescent antibody techniques (Kawahara et al. 1986).

8. Epizootiology

Reservoir of infection

Enterococci released from diseased fish seem to be the main source of infection. It has been shown that these bacteria remain in seawater and particularly in the mud around farms for a long time. Higher numbers of microorganisms exist in the seawater during summer months in contrast to the winter months when the bacterial load is higher in the mud (Kitao et al. 1979). Ghittino & Prearo (1992) have shown that in freshwater fish the outbreak of the disease was related to an organic pollution of the rivers supplying fish farms, associated with high water temperature (21-22°C).

Wild fish can also be infected and spread the bacteria. Minami (1979) recovered *Streptococcus* species from a

number of wild fish, such as anchovies (*Engraulis japonica*), sardines (*Sardinops melanosticta*), the round herring (*Etrumeus micropus*). Furthermore, it was found that the bacteria can survive in frozen fish for up to six months and that some of these fish are used as fish feed in Japanese mariculture (Minami 1979; Yasunaga 1982). Thus, contaminated feedstuff can also be a source of infection for farmed fish. Fish that survive from epizootics are also reservoirs of the infection.

9. Mode of transmission

It is generally believed that the infection is horizontal with infection occurring from direct contact with infected fish or contaminated fish food. Robinson & Meyer (1996) proved that the disease could be readily transmitted experimentally by introducing infected golden shiners to healthy aquarium fish. Infection could also be induced by placing healthy fish in bath suspensions of bacterial cells for 10 minutes. Many researchers have reported successful experimental infections by the intraperitoneal and intramuscular route (Hoshina et al. 1958, Robinson & Meyer, 1966, Cook & Lofton, 1975; Ohnishi & Jo, 1981; Sugiyama et al. 1981; Iida et al., 1986. Recently, Romalde et al. (1996), tried different routes of experimental infections in turbot and concluded that the horizontal transmissions through water and the fecal-oral route are the most important mode of infection in this fish species. A number of reports exists in the recent literature suggesting that the environmental conditions influence the transmission and pathogenicity of the disease. Perera et al. (1997) reported that greater mortality rates were observed in artificially infected tilapia (by oral administration and by dipping fish in contaminated water) maintained at 20°C than those kept at 15, 25, 30 or 35°C and in water with pH=9, compared to fish kept in more acidic water. Fukuda et al. (1997a,b) have also showed that horizontal transmission occurs more easily among yellowtail kept in hypoxic than in hyperoxic conditions.

10. Pathogenicity

The mechanisms of enterococcal infections pathogenicity are not yet fully understood. The pathogenicity of a-haemolytic *Streptococcus* species in yellowtail is enhanced by *in vivo* passage through yellowtail (Kusuda & Kimura 1978). KG-types are considered more virulent than KG+ strains (Kitao 1983). The pathogenicity of *Enterococcus seriolicida* appears to be partly, at least, related to the agglutination pattern (Alim et al. 1996) and to an exotoxin (Watson 1960), which is highly related to disease and death in this fish species (Kimura & Kusuda, 1979; Minami et al, 1979; Kusuda & Hamaguchi 1988, 1989). The virulence of *Streptococcus shiloi* and *S. difficile* in tilapia (*O. niloticus*) and trout was also increased after three passages *in vivo* without culture on agar (Eldar et al. 1995b). Robinson and Meyer (1966) found that injections of group b (gamma haemolytic) streptococci are lethal to

some marine fish species. Cook & Loften (1975) investigated the pathogenicity of the *Streptococcus* species, initially isolated by Plumb et al. (1974) to five species of fish. Ohnishi & Jo (1981) found that b-haemolytic *Streptococcus* species isolated from ayu were very pathogenic for tilapia, yellowtail and seabream (*Pagrus major*), but not for either carp or black seabream (*Acanthopagrus schlegel*).

However, recent studies on *Streptococcus* species isolated from tilapia, carp, mullet and striped hybrid bass (a-haemolytic) showed that only stress factors increased the mortality and the authors suggested that these species are opportunistic pathogens, because they are widespread in the aquaculture environment and because of their dependence on stress to assert pathogenicity (Bunch & Bejerano 1997).

11. Control

Chemotherapy and preventive measures

Erythromycin is generally believed to be effective against streptococcal infections in cultured yellowtails (Shiomitsu et al. 1980; Katae et al. 1980), in rainbow trout (Kitao et al. 1987; Carson & Statham 1993) at doses 25-50 mg/kg body weight of fish/day for 4-7 days. Erythromycin was also used to treat Gram positive infections in sea bream and bass in Greece at a dose 50-100 mg/kg body-weight without though, eradicating the disease (Varvarigos 1998). Other antibiotics like doxycycline (Kitao & Aoki 1979), kitasamycin, alkyl-trimethyl-ammonium-calcium-oxytetracycline, josamycin, oleandomycin and linkomycin have also been used to control streptococcosis in cultured yellowtail in Japan (Inglis et al. 1993; Carson & Statham 1993). Aoki et al (1990) has reported resistance of these antibiotics among strains of *Streptococcus* species isolated in Japan and Bates (1997) has documented the presence of vancomycin-resistant enterococci in the stools of asymptomatic human individuals. Enrofloxacin was recently suggested to have excellent potential as an antibacterial for sunshine bass infected with *Streptococcus iniae* (Stoffregen et al. 1996). Recently, the research is focusing in preventing the disease. Reducing overcrowding,

overfeeding, unnecessary handling or transportation and prompt removal and slaughter of all moribund fish in ponds or cages at an early stage of infection is still an effective way of preventing outbreaks. Ozone treatment of marine water was proved to be effective in disinfecting seawater from *Enterococcus seriolicida* and other pathogens and was suggested as a means of improving mariculture efficiency (Sugita et al. 1992). Furthermore, recent research is directed towards boosting the immune status of the farmed fish. New substances, such as peptidoglycans (Matsuyama et al. 1992; Itami et al. 1996; Tinman et al. 1997) and bovine lactoferrin (Sakai et al. 1993a) have been tried due to their ability to enhance resistance to rainbow trout and Japanese farmed fish. Toranzo et al. (1996) found that b-glucan in feed induced an enhancement of the non-specific defense mechanisms of turbot, but did not protect it against *Enterococcus* infection. Vitamins, like tocopherol and mineral mixtures, have also been tested in relation to resistance to streptococcal disease in yellowtails, but the authors found that fish infected with *Streptococcus* sp. had strong resistance with increased survival rate, regardless to the diets used (Sekiya et al. 1991).

Vaccine development/immunization

Iida et al. (1986) and Sakai et al. (1987, 1991, 1993a) were the first researchers to report success with bacterins, which provided protection against the challenge of artificial infection. Since then, efforts have been directed towards the immunological protection against enterococcosis both by antigenic immunization and enhancement of the immune system with immunostimulants and by vaccine development. A number of reports suggest that vaccination would be the solution in combating Gram positive infections in farmed fish, but so far no commercially available vaccine exists (Ghittino et al 1995). Eldar et al. (1995b, 1997), Toranzo et al. (1995), Akhlaghi et al. (1996), Kusuda et al. (1996), Leiro et al. (1996) and Bercovier et al. (1997) have demonstrated the efficacy of intraperitoneal infections of formalized bacterins of *Enterococcus* strains for protection from autologous infections in different fish species. □

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