24

Antimicrobial Resistance and Potential Probiotic Application of *Enterococcus* spp. in Sea Bass and Sea Bream Aquaculture

Ouissal Chahad Bourouni[1], Monia El Bour[1],
Pilar Calo-Mata[2] and Jorge Barros-Velàzquez[2]
[1]*Institut National des Sciences et Technologies de la Mer (INSTM), Tunis,*
[2]*Department of Analytical Chemistry, Nutrition and Food Science, LHICA,*
School of Veterinary Sciences, University of Santiago de Compostela,
[1]*Tunisia*
[2]*Spain*

1. Introduction

Microbial resistance to antibiotics is a world-wide problem in human and veterinary medicine. It is generally accepted that the main risk factor for the increase in the antibiotic resistance is an extensive use of antibiotics. In fact, for the last 50 years, high levels of antibiotics are commonly used for treatment and prevention of infectious diseases in humans and animals. This led to emergence and dissemination of resistant bacteria and resistance genes in wild populations (Bogaard & Stobberingh 2000). The antimicrobial agents used in animal care are also significant, both in increasing resistance in animal pathogens, and in transmission of resistant bacteria from animals to humans. In part, this is due to the transfer of antimicrobial-resistant normal or commensal microflora of animals, *via* the food chain to humans. Several recent papers reported link between antibiotic use in food producing animals, emergence of antibiotic resistance in *Salmonella*, *Escherichia coli*, enterococci or *Campylobacter* in treated animals and transfer of these resistances to humans (or their resistance genes to human pathogens) *via* the food chain (Barton 2000; Angulo et al. 2004). However, less attention was paid to potential for antibiotic use in aquaculture industries to compromise human health. In addition to transfer of resistant bacteria through consumption of contaminated fish and shellfish, there is substantial risk of environmental contamination due to practice of using medicated feeds to treat whole pens or cages.

2. Antibiotic resistance in aquaculture

Aquaculture around the Mediterranean basin has increased significantly to satisfy the demand for seafood, which cannot be met by wild fisheries harvesting as this is currently in a state of decline because of over-fishing, pollution and marine habitat destruction. Recent reports of the United Nations Food and Agriculture Organization (FAO), noted approximately more than 290.10^3 tons for the mainly species of marine fish farmed (sea bass and sea bream) and had previously estimated that half of the world's seafood demand will

be met by aquaculture in 2020 (FAO, 2008). In Mediterranean aquaculture, the culture practices for most farmed fish species are mostly semi-intensive or intensive and a significant challenge to fish farming however is disease caused by bacteria such as *Aeromonas* sp., *Vibrio* sp., *Pseudomonas* sp. and *Flavobacterium* sp. Both prophylactic and therapeutic treatments utilize drug supplemented feeds to keep farmed fish free of diseases.

Antibiotics such as oxytetracycline (OTC) and quinolone such as oxolinic acid (OA) are the most widely used in Mediterranean aquaculture in feed (Rigos & Troisi, 2005) and treatments discharge drugs directly into the marine environment, where they are relatively resistant to biodegradation. Rigos et al., 2004 found that 60-73% of the OTC and 8-12% of the OA administered to farmed sea bream were excreted with the faeces. Also, the results of ARMed (Antibiotic Resistance in the south-eastern Mediterranean) suggest existence of high resistances of bacteria particularly in the eastern region where the resistance in *E. coli* appears to be more important than in other Mediterranean countries.

Previous reports noted that resistance emergence result directly from infections treatment with antibacterial drugs (Sorum 1998, 1999) and therefore limited their value in control of bacterial diseases of fish (Smith et al., 1994), apart from any public health concerns.

Further, antibacterial drugs were shown to persist in animal tissues and in the sea, including the aquatic food chain (CIESM, 2004) and development of antibiotic resistance is direct consequence of drug pollution. Chelossi et al., 2003 found that antibiotics discharged through faeces or undigested feed, contributed to high incidences of quinolone, tetracyclin and penicillin-resistant benthic bacteria and caused a shift in structure of the benthic microbial assemblage next to fish farms. Moreover, a considerable increase in resistance to several antimicrobial drugs has been discovered in some species of *Vibrio* and *Pseudomonas* recovered from diseased farmed sea bream of south-western Spain (Zorilla et al., 2003).

In Turkey, bacteria isolated from sea bass (*Dicentrarchus labrax*) showed a multidrug resistance to trimethoprim-sulfamethoxazole, cephalothin, tetracyclin and streptomycin suggesting that fish farms act as a reservoir of multidrug-resistant pathogenic bacteria such as *Pseudomonas* and *Vibrio* (Matyar, 2007). Considering the frequent usage of anti-bacterial drugs in Mediterranean fish farming, and serious problems of their rapid increase in resistance and transfer to non-target microflora including human and animal pathogens, there is an urgent need for monitoring drug contamination in aquatic environment and thus, the need for alternative techniques replacing drugs with effective and inexpensive probiotics which became increasingly evident and necessary to avoid resistance in fish farming sites and antibiotic residues in fish flesh destined for human consumption.

In Tunisia, aquaculture fish industry was developed since 1989 and has highly increased during these last ten years and national production passed from 1566 tons in 2000 to 4468 tons in 2009 with an increase in number of aquatic farms multiplied by about five. The production statistics in 2009 noted more than 2800 tons for marine fish farming. Regarded as a strategic activity that can support the fishing sector, aquaculture benefits in Tunisia of a particular interest mainly for the most two marine species farmed sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*), which were undertaken in almost private farms.

The evolution of antibiotic resistance of the main bacterial species of medical interest is subject to increased surveillance in Tunisia. Since 1999, the research laboratory on antibiotic

resistance (LAB MDT-03) established a system for monitoring bacterial resistance to antibiotics (L'Antibio - Résistance en Tunisie or LART). It includes four hospitals regularly monitoring the epidemiology of major bacterial species of medical importance and antibiotic resistance data collected are used in development of recommendations to antibiotic therapy (Boutiba et al., 2007). However, the problem of antibiotic resistance is underestimated in animal production including aquaculture and studies related are scarced. A study of pathogens vibrios isolated from sea bass showed a multi-resistance of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* for almost antibiotics used and sensitivity was only demonstrated for furazolidone and chloramphenicol (Bakhrouf et al., 1995). Bouamama et al., 2001 isolated several multiresistant bacteria from mussel *Mytilus galloprovincialis* with resistance profiles to 12 different antibiotics in *Aeromonas hydrophila* and *Propioni acnes*. *Vibrio alginolyticus* was isolated from internal organs of sea bream and sea bass reared in two fish farms located in Tunisian coast. Multi-drug resistance to antimicrobial agents was detected, all the 34 strains tested were resistant to ampicillin, 31 strains were resistant to nitrofurantoïne and 12 were resistant to tetracycline (Ben Kahla-Nakbi et al., 2006). The most recent study of Rezgui et al., (2010) showed abundance of antibiotic resistant bacteria isolated mainly from gills and intestinal tract of sea bre*am* and sea bass which belong to several species of the genus *Pseudomonas, Aeromonas, Vibrio* and *Enterobacteriaceae* and were resistant essentially to tetracyclin and penicillin (antibiotics commonly used respectively in veterinary and human clinical).

3. Probiotics as alternative to antibiotics in aquaculture

The increasing problems associated with infectious diseases in fish, the frequent usage of drugs for treatment and prevention of these diseases and the rapid increase in resistance to these antibiotics represent major challenges for this source of food production worldwide. Thus, replacing drugs with effective and inexpensive probiotics was became increasingly evident and necessary to avoid resistance in fish farming sites and antibiotic residues in fish flesh destined for human consumption (Vershuere et al., 2000; Balcazar et al., 2006; Rengpipat et al., 2008).

3.1 Probiotics: definition and principles

The term, probiotic, simply means "for life", originating from the Greek words "pro" and "bios" (Gismondo et al., 1999). The most widely quoted definition was made by Fuller (1989). He defined a probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". This definition is still widely referred to, despite continual contention with regard to the correct definition of the term. Current probiotic applications and scientific data on mechanisms of action indicate that non-viable microbial components act in a beneficial manner and this benefit is not limited just to the intestinal region (Salminen et al., 1999). Besides, based on the intricate relationship an aquatic organism has with the external environment when compared with that of terrestrial animals, the definition of a probiotic for aquatic environments needs to be modified. Verschuere et al. (2000a) suggested the definition "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment".

3.2 Different modes of action

Several studies have demonstrated certain modes of probiotic action in effect in the aquatic environment. Bairagi et al. (2002) assessed aerobic bacteria associated with the gastrointestinal tract (GIT) of nine freshwater fish. They determined that selected strains produced digestive enzymes, thus facilitating feed utilization and digestion. Ramirez & Dixon (2003) reported on the enzymatic properties of anaerobic intestinal bacteria isolated from three fish species, showing the potential role a probiotic could play. In the paper of Bairagi et al. (2004) the benefit of adding *B. subtilis* and *B. circulans* to the diet of rohu, *Labeo rohita*, was shown. In the search to replace fish meal with leaf meal in fish feed, they found that addition of the two fish intestinal *Bacillus spp*. increased performance as judged by several factors (growth, feed conversion ratio, and protein efficiency ratio). They attributed this to the extracellular cellulolytic and amylolytic enzyme production by the bacteria. Although competition for adhesion sites has been widely suggested as a mode of action, there is little evidence in the literature to demonstrate this. Although for not direct attachment competition, Yan et al. (2002) demonstrated that production of antibiotic substances by two seaweed-associated *Bacillus* sp. was dependent on biofilm formation by the bacteria. This study highlighted a factor which might be important for some bacteria to be effective probiotics, i.e. surface attachment. Such observation concurred with Fuller's (1989) definition of a probiotic, i.e. the requirement for GIT colonization. It has been proposed that the mechanism of competitive exclusion for attachment sites could be given a distinct advantage via addition of probiotic bacteria during the initial egg fertilization steps of larviculture, thereby "getting in there first" (Irianto and Austin, 2002a).

Several studies have attributed a probiotic effect to competition for energy sources (Rico Mora et al., 1998; Verschuere et al., 1999; Verschuere et al., 2000b). Beneficial growth and survival was found in Artemia sp. pre-exposed to nine strains of bacteria before challenge with *V. proteolyticus* (Verschuere et al., 1999). It was concluded that the effect was not caused by extracellular products, but required the live bacterial cell. Although it was not specifically tested, they hypothesized that the protective effect probably resulted from competition for energy sources and for adhesion sites.

Itami et al. (1998) found that addition of *Bifidobacterium thermophilum* derived peptidoglycan to kuruma shrimp increased significantly their survival when they were challenged with *V. penaeicida*. They attributed this to an immunostimulatory effect, as the phagocytic activity of shrimp granulocytes was significantly higher in the treated shrimp compared with those of the control animals. Gullian et al. (2004) tested immunostimulation by a live *Vibrio sp*. (P62) and *Bacillus sp*. (P64), using *V. alginolyticus* as a positive control. They concluded that P64 and *V. alginolyticus* were immunostimulants. A review by Smith et al. (2003) provided important information on the potential problems associated with immunostimulants in crustacean aquaculture. They argued that the prolonged use of immunostimulants was in fact detrimental to the host and that much more research was needed before their use during critical periods could be considered safe.

Competition for iron has been reported as an important factor in marine bacteria (Verschuere et al., 2000a). Iron is needed by most bacteria for growth, but is generally limited in the tissues and body fluids of animals and in the insoluble ferric Fe3+ form (Verschuere et al., 2000a). Iron-binding agents, siderophores, allow acquisition of iron suitable for microbial growth. Siderophore production is a mechanism of virulence in some

pathogens (Gram et al., 1999). Equally, a siderophore producing probiotic could deprive potential pathogens of iron under iron limiting conditions. This was shown by Gram et al. (1999), who found that a culture supernatant of *Pseudomonas fluorescens*, grown in iron-limited conditions, inhibited growth of *V. anguillarum*, whereas the supernatant from iron-available cultures did not.

Possibly the most studied mode of probiotic action in aquatic animals is the production of inhibitory substances. Currently, there are four methods commonly employed to screen for inhibitory substances *in vitro*; the double layer method, the well diffusion method, the cross-streak method, and the disc diffusion method. All methods are based on the principle that a bacterium produces extracellular substance inhibitor to itself or another bacterial strain (the indicator). The inhibitory activity is displayed by growth increase of the producer culture in agar medium.

This *in vitro* screening method has identified very good probiotics in aquaculture (Irianto & Austin, 2002b; Lategan and Gibson, 2003; Vaseeharan et al., 2004; Lategan et al., 2004a,b), with two major limitations for this approach. The first is that other modes of probiotic activity (e.g. immunostimulation, digestive enzymes production, competition for attachment site, or nutrients) will not be expressed in the laboratory on agar plate and, hence, a major source of potential beneficial action will be overlooked. The second drawback is that positive results *in vitro* fail to determine the real *in vivo* effect.

3.3 Developing probiotics for aquaculture

It has been widely published that a probiotic must possess certain properties (Verschuere et al., 2000a). These properties were proposed in order to aid in correct establishment of new, effective and safe products and included:

1. The probiotic should not be harmful to the host it is desired for,
2. It should be accepted by the host, e.g. through ingestion and potential colonization and replication within the host,
3. It should reach the location where the effect is required to take place,
4. It should actually work *in vivo* as opposed to *in vitro* findings,
5. It should preferably not contain virulence resistance genes or antibiotic resistance genes.

The future application for probiotics in aquaculture looks bright. There is an ever-increasing demand for aquaculture products and a similar increase in the search for alternatives to antibiotics. The field of probiotics intended for aquacultured animals is now attracting considerable attention and a number of commercial products are available.

3.4 Probiotic strains studied in aquaculture

Most probiotics proposed as biological control agents in aquaculture belong to the lactic acid bacteria (*Lactobacillus* and *Carnobacterium*), although other genera or species have also been studied, belonging to the genus *Vibrio*, to the genus *Bacillus*, or to the genus *Pseudomonas*, and also *Aeromonas* and *Flavobacterium* (Table 1).

Within probiotic group, lactic acid bacteria (LAB) have been recognized for their fermentative ability as well as their health and nutritional benefits since they exert strong

antimicrobial activities against many pathogenic microorganisms and were considered as harmless bacteriocin-producing strains which may act antagonistic against fish pathogens (Maugin & Novel, 1994; Ringo & Gatesoupe, 1998). Moreover, LAB were signalled as competing for nutrients or space with spoiling microorganisms due to their ability to produce organic acids, hydrogen peroxide, diacetyl and bacteriocins and therefore should be of applied interest for marine fish and shellfish food bio-preservation (Franz C. et al., 2007).

Animals tested	Potential probiotic	Pathogen tested or type of study conducted	Test method
Gilthead sea bream	*Cytophaga sp., Roseobacter sp., Ruergeria sp., Paracoccus sp.,A. sp., Shewanella sp.*	Natural larval survival study	*In vivo*
Gilthead sea bream	*V. spp., Micrococcus sp.*	*L. anguillarum*	*In vitro and in vivo*
Atlantic cod	*Carnobacterium divergens*	*V. anguillarum*	*In vitro and in vivo*
Atlantic cod	*Carnobacterium divergens*	*V. anguillarum*	*In vitro and in vivo*
Atlantic salmon	*Lactobacillus plantarum*	*A. salmonicida*	*In vitro and in vivo*
Atlantic salmon	*Carnobacterium sp. (K1)*	*V. anguillarum, A. salmonicida*	*In vitro and in vivo*
Atlantic salmon	*Ps. fluorescens*	*A. salmonicida*	*In vitro and in vivo*
Atlantic salmon, rainbow trout	*Carnobacterium sp.*	*V. anguillarum, V. ordalii, Y. ruckeri, A. salmonicida*	*In vitro and in vivo*
Eel	*Commercial product: Cernivet® LBC (Ent. Faecium SF68), Toyocerin® (B. toyoi)*	*Ed. tarda*	*In vivo*
Eel.	*A. media*	*Saprolegnia sp*	*In vitro and in vivo*
Eel	*A. media*	*Saprolegnia parasitica*	*In vivo*
Goldfish	*Dead cells of A. hydrophila*	*A. salmonicida*	*In vivo*
Indian major carp	*B. subtilis*	*A. hydrophila*	*In vivo*
Nile tilapia	*Str. faecium, Lactobacillus acidophilus, Sacc. cerevisiae*	*Growth study*	*In vivo*
Pollack	*Commercial product: Bactocell (Pediococcus acidilactici), Levucell (Sacc. cerevisiae)*	*Pollack growth study using enriched Artemia*	*In vivo*
Rainbow trout	*Ps.fluorescens*	*V. anguillarum*	*In vitro and in vivo*
Rainbow trout	*Lactobacillus rhamnosus*	*A. salmonicida ssp. salmonicida (furunculosis)*	-
Rainbow trout	*Ps. spp.*	*V. anguillarum*	*In vitro and in vivo*
Rainbow trout	*A. hydrophila, V. fluvialis, Carnobacterium sp.*	*A. salmonicida*	*In vitro and in vivo*
Rainbow trout	*Dead cells of A. hydrophila, V. fluvialis, Carnobacterium sp.*	*A. salmonicida*	*In vivo*
Rainbow trout	*Lactobacillus rhamnosus*	*Immune enhancement paper*	*In vivo*
Rainbow trout	*Commercial product: BioPlus2B (B. subtilis, B. licheniformis)*	*Y. ruckeri*	*In vivo*
Rainbow trout	*Lactobacillus rhamnosus*	*Natural immunostimulation measured*	*In vivo*
Rainbow trout	*Pediococcus acidilactici, Sacc. boulardii*	*Prevention of vertebral column compression syndrome*	*In vivo*
Rainbow trout	*A. sobria*	*L. garvieae, Str. iniae*	*In vivo*
Rainbow trout	*Lactobacillus rhamnosus*	*Natural immunostimulation measured*	*In vivo*
Rohu	*B. circulans, B. subtilis*	*Digestive enzyme study*	*In vivo*
Sea bass	*Debaryomyces hansenii, Sacc. cerevisiae*	*Digestive enzyme study*	*In vivo*
Senegalese sole	*V. spp., Ps. spp., Micrococcus sp.*	*V. harveyi*	*In vitro and in vivo*
Silver perch	*A. media*	*Saprolegnia sp.*	*In vivo*
Tilapia	*Commercial product: Alchem Poseidon, Korea*	*Ed. tarda*	*In vivo*
Turbot	*2 unidentified marine bacteria*	*GIT colonization study*	*In vivo*
Turbot	*Marine bacteria*	*Natural survival study*	*In vivo*
Turbot	*Roseobacter spp., V. spp.*	*V. anguillarum, V. splendidus, Psalt. sp.*	*In vitro and in vivo*

Table 1. Summary of research towards probiotics for finfish

The LAB bacteriocin producer widespread in nature, and were isolated from several sources: dairy products, fermented sausages, vegetables, sillage, and mammalian gastro-intestinal tract (Laukova et al., 1993; Kato et al., 1994; Giraffa, 1995; Ennahar et al.,1998). Recently, bacteriocin producing LAB were efficiently tested in attempt to improve aquatic

environment for both shrimp and fish aquaculture (Calo-Mata P et al., 2007; Chae-Woo et al., 2009). Among them, bacteria belonging to the genus *Enterococcus* are primarily associated with the indigenous human and animal gastrointestinal flora and are widely distributed, being found in air, water, sewage, soil and vegetation (J. Lukasova & A. sustackova, 2003). Although certain *Enterococcus* spp. have recently been associated with human nosocomial infections (Murray, 1998), a wide variety of enterococcal strains are increasingly being used as probiotics owing to their contribution to the healthy microflora of human mucosal surfaces. They have also been introduced into animal foods owing to their contribution to the health of farmed animals and as biological control agents in aquaculture (Calo-Mata P. et al., 2007).

4. Probiotic development in aquaculture farming in Tunisia

In Tunisia, fish farming of the two species sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) has significantly increased since a great benefit for such aquaculture which was threatened by microbial infections causing high mortalities at larval stages, and therefore decrease in farmed fish production. In addition, widespread use of antibiotics created an ecological problem for coastal ecosystems due to emergence of antibiotic resistant pathogen bacteria (Bouamama, 2001; Dellali, 2001; El Bour et al., 2001). Therefore, selection and use of probiotic bacteria capable of inhibiting pathogenic bacteria in sustainable way without ecosystem alteration would be a useful for specific farming problems. In this scope, for several years the INSTM team in Tunisia, in collaboration with the Department of Analytical Chemistry, Nutrition and Food Science, from the University of Santiago de Compostela in Spain were focusing in isolation and characterization of probiotic group, lactic acid bacteria (LAB) which were recognized for their fermentative ability as well as their health and nutritional benefits.

The study aimed to investigate the occurrence and antibiotic resistance profiles of *Enterococcus* spp. associated to the skin and the gastrointestinal tract of farmed sea bass and sea bream, the main fish species with high economic value cultured in Mediterranean aquaculture. This was accomplished by phenotypic and genotypic analysis, the latter including 16S rRNA sequencing and RAPD-PCR analysis. Besides, and with a view to perform a preliminary screening of potential probiotic LAB, the strains were investigated in their ability to produce antibacterial compounds against spoilage and pathogenic bacteria.

4.1 Methodology

Gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) were collected from a fish farm in Hergla (Aquaculture Tunisiènne, Monastir, Tunisia). Skin patches were excised and the intestinal content was removed by dissecting the fish, removing the intestine and squeezing out the contents. Eighty four LAB strains were then isolated and investigated. The phenotypic characterization of bacterial isolates was studied to determine their colony morphology, cell morphology, motility, Gram stain and the production of cytochrome oxidase and catalase. The phenotypic identification of LAB strains was carried out by means of miniaturized API 50 CH biochemical tests (BioMérieux, Marcy L'Etoile, France). The results of the identification tests were interpreted using the APILAB PLUS software (BioMérieux).

Production of antibacterial activities was investigated, against a range of 39 pathogenic and spoilage microorganisms (Table2), to select potential producer strains. Detection of bacteriocin activity in LAB strains was screened by means of a standardized agar disk diffusion method.

Code	Genus	Species	Origin
AmH01	*Aeromonas*	*hydrophila*	ATCC 7966
BaC23	*Bacillus*	*cereus*	ATCC 14893
BaP31	*Bacillus*	*pumilus*	ATCC 7061
BaS05	*Bacillus*	*Subtilis ssp. Spizizenii*	ATCC 6633
BxT01	*Brochotrix*	*thermosphacta*	ATCC 11509
CbD21	*Carnobacterium*	*divergens*	ATCC 35677
CbM01	*Carnobacterium*	*maltaromaticum*	LHICA collection
EbA01	*Enterobacter*	*aerogenes*	ATCC 13048
EbC11	*Enterobacter*	*cloacae*	ATCC 13047
HaA02	*Hafnia*	*alvei*	ATCC 9760
KlOx11	*Klebsiella*	*oxytoca*	ATCC 13182
KlP02	*Klebsiella*	*planticola*	ATCC 33531
KlPn21	*Klebsiella*	*Pneumoniae* ssp. *pneumoniae*	ATCC 10031
Lb30A	*Lactobacillus*	*saerimneri*	LHICA collection
MoM02	*Morganella*	*morganii* ssp. *morganii*	ATCC 8076H
PhD11	*Photobacterium*	*damselae*	ATCC 33539
PrM01	*Proteus*	*mirabilis*	ATCC 14153
PrP11	*Proteus*	*penneri*	ATCC 33519
PrV21	*Proteus*	*vulgaris*	ATCC 9484
PsF12	*Pseudomonas*	*fluorescens*	ATCC 13525
PsFr51	*Pseudomonas*	*fragi*	ATCC 4973
PsG21	*Pseudomonas*	*gessardii*	LHICA collection
SrM53	*Serratia*	*marcescens* ssp. *marcescens*	ATCC 274
SyE21	*Staphylococcus*	*epidermidis*	ATCC 35983
SyX11	*Staphylococcus*	*xylosus*	ATCC 29971
StM03	*Stenotrophomonas*	*maltophilia*	ATCC 13637
59	*Staphylococcus*	*aureus*	ATCC 9144
4521	*Staphylococcus*	*aureus*	ATCC 35845
4032	*Lysteria*	*monocytogenes*	NCTC 11994
1112	*Lysteria*	*monocytogenes* 1112	LHICA collection
CI34.1	*Pseudomonas*	*anguilliseptica*	Seabream*
ACR5.1(AS)	*Aeromonas*	*salmonicida*	Turbot*
CI52.1(VCI)	*Vibrio*	*anguillarum*	Seabream*
ACC30.1	*Photobacterium*	*damselae* ssp. *piscida*	Sole*
V62	*Vibrio*	*anguillarum*	Seabream**
VF	*Vibrio*	*anguillarum*	Seabass***
AF	*Aeromonas*	*salmonicida*	Seabass***
V90.11.287(V287)	*Vibrio*	*anguillarum*	Seabass****
AH2	*Pseudomonas*	*fluorescens*	*Lates niloticus*****

* Strains provided by Pr. J. L. Romalde (Spain). ** Strain provided by Pr. G. Breuil (France).
*** Strains provided by Pr. J. C. Raymond (France). **** Strains provided by Pr. L. Gram (Denmark).

Table 2. Pathogenic and spoilage indicator microorganisms used to test the antibacterial activities of LAB isolates.

Genetic characterization of producer LAB strains was then performed by PCR targeted to the 16S rRNA gene using the universal set of primers: p8FPL (forward: 5′-AGTTTGATCCTGGCTCAG-3′) and p806R (reverse: 5′-GGACTACCAGGGTATCTAAT-3′), that yield a 800 bp PCR product of the 16S rRNA gene. The PCR products were purified and sequenced. The sequences were compared with others present in GenBank database.

Further genetic characterization of LAB isolates was performed by RAPD-PCR using primers M13 (5´-GAGGGTGGCGGTTCT-3´) (Andrighetto et al., 2004). To check reproducibility, all PCR assays were performed in triplicate. Each reaction we included a tube without template DNA as a negative control.

The antibacterial sensitivity was determined by the agar diffusion method according to Chabbert (1982), using 16 antibiotics that were selected as representative of different classes of antimicrobial agents relevant in human and animal medicine (Penicillin G, Amoxicillin, Oxacilin, Cefoxitin, Ceftriaxon, Streptomycin, Tobramycin, Neomycin, Chloramphenicol, Tetracyclin, Oleandomycin, Nitrofurantoin, Trimethoprim-Sulphonamid, Rifampicin, Oxolinic acid and also Vancomycin). Based on the zones of inhibition a qualitative report of "susceptible", "intermediate" or "resistant" can be determined for the tested bacteria according to French national guidelines (Comité de l'Antibiogramme de la Société Française de Microbiologie, 1996).

4.2 Results and discussion

Eighty four strains of LAB were isolated from both gastrointestinal content and skin of fish studied. All isolates were Gram-positive, catalase-negative, facultatively anaerobic and nonmotile chain-forming cocci. They were tested for assaying inhibitory production against 39 Gram-positives and Gram-negatives bacteria, including pathogenic bacteria in aquaculture and others spoilage bacteria. 58 strains (69%) exhibited inhibitory activity against a large number of the indicator organisms investigated. Greater inhibition was observed against *L. monocytogenes, S. aureus*, *A. hydrophila, A. salmonicida, V. anguillarum* and *Carnobacterium* strains in comparison with the remaining indicators. The diameters of the inhibition halos were within the 7.5–18 mm range. Thus, we selected 35 highly producing strains that generated inhibitory zones with diameters between 12 and 18 mm.

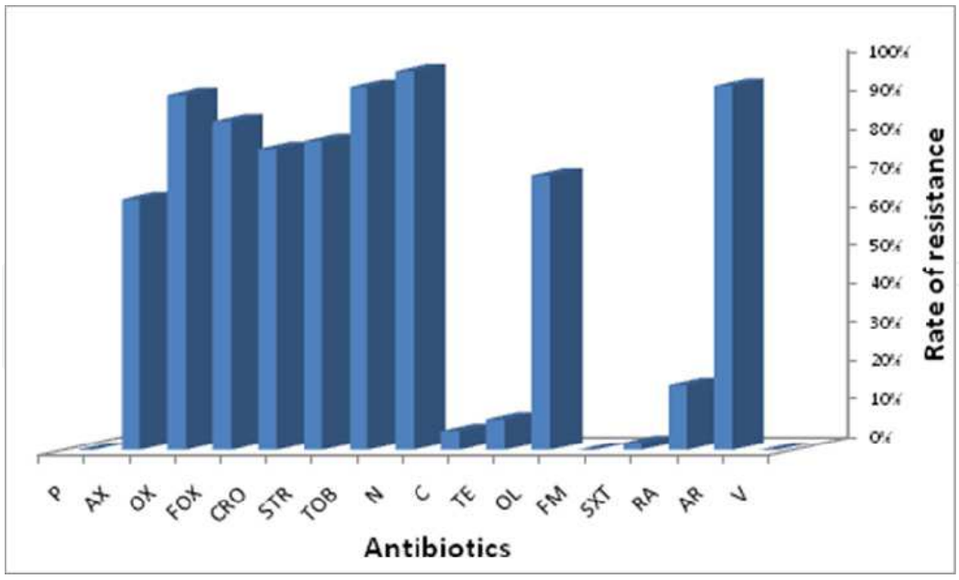
The results allowed the classification of the strains as belonging to the species *E. faecium* (29 strains) and *E. sanguinicola* (6 strains) (paper under process) (Table3). Other studies, previously mentioned, also showed that the skin and gastrointestinal tract of various fish species contains lactic acid bacteria which produce antibacterial compounds able to inhibit the growth of several micoorganisms (Ringo 1999; Spanggaard B. et al., 2001; Rengpipat S. et al., 2008; Vijayabaskar P & Somasundaram S. T., 2008; Ringo, 2008).

According to the results obtained, all the strains tested were resistant to at least three different antibiotics. The frequency of resistance to the various antimicrobials for all bacteria is presented in Fig. 1. Differences of resistance rates were noted for amoxicillin, oxacillin, cephalosporins (cefoxitin, ceftriaxon), aminosids (streptomycin, tobramycin and neomycin), macrolids (oleandomycin) and oxolinic acid. In contrast, phenicol, tetracyclin, rifampicin and trimethoprim-sulphamid were the most active antibiotics against the majority of the bacterial isolates (fig1).

In fact, more than half (64.8%) of all the isolates were found to be resistant to amoxicillin and 71.4% were resistant to oleandomycin, 78.1% to ceftriaxon, 80.1% to streptomycin and 85% to cefoxitin. Oxacillin resistance was found in 92.5% of the isolates and tobramycin and oxolinic acid resistance in 94.2%. Resistance to neomycin was found in 98.3% of the isolates. Resistance to chloramphenicol and trimethoprim-sulphamide was detected in 5.1% and 2.2% of the isolates respectively, 8% of the isolates were found to be resistant to tetracyclin. Resistance to rifampicin was seen in 16.9% of the isolates

Strains	Fish	Organ	Identification	Accession number
UPAA 1	Sea bass	GIT	*Enterococcus sanguinicola*	GU460379
UPAA 4	Sea bass	GIT	*Enterococcus faecium*	GU460381
UPAA 7	Sea bass	Skin	*Enterococcus faecium*	GU460383
UPAA 11	Sea bream	GIT	*Enterococcus faecium*	HQ450696
UPAA 15	Sea bass	Skin	*Enterococcus faecium*	GU460385
UPAA 23	Sea bass	GIT	*Enterococcus faecium*	GU460388
UPAA 24	Sea bass	GIT	*Enterococcus faecium*	GU460389
UPAA 25	Sea bass	GIT	*Enterococcus faecium*	GU460390
UPAA 31	Sea bass	Skin	*Enterococcus faecium*	GU460394
UPAA 32	Sea bass	GIT	*Enterococcus faecium*	GU460395
UPAA 33	Sea bass	GIT	*Enterococcus sanguinicola*	GU460396
UPAA 34	Sea bream	Skin	*Enterococcus faecium*	HQ450701
UPAA 35	Sea bream	GIT	*Enterococcus faecium*	HQ450702
UPAA 37	Sea bass	GIT	*Enterococcus faecium*	GU460398
UPAA 39	Sea bream	GIT	*Enterococcus faecium*	HQ450704
UPAA 40	Sea bream	GIT	*Enterococcus faecium*	HQ450705
UPAA 44	Sea bream	Skin	*Enterococcus faecuim*	HQ450706
UPAA 45	Sea bream	GIT	*Enterococcus faecuim*	HQ450707
UPAA 53	Sea bass	GIT	*Enterococcus faecium*	GU460402
UPAA 54	Sea bass	GIT	*Enterococcus faecium*	GU460403
UPAA 56	Sea bass	Skin	*Enterococcus faecium*	GU460404
UPAA 57	Sea bass	Skin	*Enterococcus sanguinicola*	GU460405
UPAA 58	Sea bass	Skin	*Enterococcus faecium*	GU460406
UPAA 63	Sea bass	GIT	*Enterococcus faecium*	GU460409
UPAA 71	Sea bream	GIT	*Enterococcus sanguinicola*	HQ450716
UPAA 80	Sea bass	GIT	*Enterococcus faecium*	GU460416
UPAA 83	Sea bass	Skin	*Enterococcus faecium*	GU460415
UPAA 85	Sea bream	GIT	*Enterococcus faecium*	HQ450721
UPAA 89	Sea bream	GIT	*Enterococcus faecium*	HQ450724
UPAA 105	Sea bass	GIT	*Enterococcus faecium*	GU460417
UPAA 110	Sea bream	GIT	*Enterococcus faecium*	HQ450730
UPAA 111	Sea bass	Skin	*Enterococcus faecium*	GU460420
UPAA 113	Sea bass	GIT	*Enterococcus sanguinicola*	GU460421
UPAA 114	Sea bass	GIT	*Enterococcus faecium*	GU460422
UPAA 116	Sea bass	Skin	*Enterococcus sanguinicola.*	GU460423

Table 3. Antibacterial producing isolates



P: penicillin G; Ax: amoxicillin; Ox: oxacillin; Fox: cefoxitin; Cro: ceftriaxon; Str: streptomycin; Tob: tobramycin; N: neomycin; C: chloramphenicol; Te: tetracyclin; Ol: oleandomycin; Fm: furans; Sxt: trimethoprim-sulphamide; Ra: rifampicin; Ar: oxolinic acid; V: vancomycine.

Fig. 1. Profiles of resistance obtained for the different enterococci isolates against the 16 antimicrobial agents tested.

Interestingly, all the strains were sensitive to vancomycin, penicillin and furans and were not haemolytic.

Enterococci have been known to be resistant to most antibiotics used in clinical practice. Multidrug-resistant and vancomycin-resistant enterococci are commonly isolated from humans, animal sources, aquatic habitats, agricultural run-off which indicates their ability to enter the human food chain (Rice et al., 1995). They are naturally resistant to cephalosporins, aminoglycosides and clindamycin and may also be resistant to tetracyclins and erythromycin. They are intermediate sensitive to penicillin and ampicillin and glycopeptides. The strains that produce β-lactamase are rare and Vancomycin-resistant enterococci (VRE) are emerging as a global threat to public health.

Enterococci are known to acquire antibiotic resistance with relative ease and to be able to spread these resistance genes to other species (Kuhn et al., 2000). *Enterococcus faecalis* has been reported to transfer plasmids harbouring antibiotic-resistance traits to other enterococci and to *Listeria monocytogenes* in water treatment plants (Marcinek et al., 1998). *Enterococcus faecium* conjugative transposons can be transferred from animal bacteria to human ones. Such conjugative trasposons can also transfer vancomycin resistance to *Staphylococcus aureus*, streptococci and lactobacilli.

The extremely high level of antibiotic resistance observed in these bacteria has made them feared infectious agents in intensive care wards. Possible pathogenicity factors like hemolysins have been described. The most important species are *E. faecalis* and *E. faecium*, the first being more common in human illnesses, the second one (though less common in human infections) may pose a larger resistance threat (Huycke et al., 1998).

In both species, the evolutionary development of resistance has been attributed to the possession of broad host range and extremely mobile genetic elements like conjugative plasmids and transposons. The molecular details of the structures and functions of these elements are fairly well studied and becoming understood (Clewell et al., 1995; Marra & Scott 1999). It is noteworthy that transcription of the transfer functions of Tn*916* requiring excission of the element is dramatically increased in the presence of tetracyclin (Celli & Trieu-Cuot 1998).

Therefore, antibiotic resistance, notably to vancomycin, and the presence of haemolysins as an indicator of potential pathogenecity, must be evaluated in these microorganisms, before they can be used as probiotics and/or food additives.

The antimicrobial spectra observed for the *Enterococcus* species isolated included several genera indicating a broad spectrum of activity against Gram-positive but also Gram-negative pathogenic and spoilage organisms. A number of earlier studies have also shown that several marine bacteria produce inhibitory substances that inhibit bacterial pathogens in aquaculture systems (Nogami & Maeda, 1992; Austin et al., 1995; Rengpipat et al., 1998; Gram et al., 1999; Chahad et al., 2007). The use of such bacteria to inhibit pathogens by release of antimicrobial substances is now gaining importance in fish farming as a better and more effective alternative than administering antibiotics to manage the health of these organisms (Vijayan et al., 2006). Spanggaard et al. (2001) reported that this antagonism was the most influential factor preventing the establishment of the exogenous bacteria and indicates that the antagonistic part of an indigenous flora may offer a significant contribution to the control of unwanted (pathogenic) bacteria.

LAB isolated from the same environment on which they will be further used as bio-control cultures, ensure that these LAB strains are well ecological adapted. This fact is an important factor for their effectiveness as natural antimicrobial agents. The local results suggest the potential usefulness of the inhibitory-producing strains isolated from fish, as probiotics in aquaculture, in order to prevent bacterial infections caused by *A. salmonicida, A. hydrophila* and *V. anguillarum* which are the most common pathogenic bacteria isolated from the marine environment, causing high mortalities of fish and shellfish. Their inhibitory activities show some properties which make it potentially remarkable food preservatives.

5. Conclusion

In comparison with studies on impact of antibio-resistance on terrestrial food producing animals, those related to marine aquaculture enterprises still scarced. The present study supports the view that there is a risk of transfer of resistant bacteria to humans from consumption of aquaculture products. In Tunisian field, although there are no products registered for use in aquaculture, antimicrobial resistance resistance is present in isolates from aquaculture..

The extent of the resistance found and in particular the significant levels of multiple resistance are of concern. Follow-up studies are required to investigate the extent of antibiotic use in Tunisian aquaculture farms and environments and to determine the molecular basis of antimicrobial resistance to the different antibiotics, the potential for transfer of resistance genes from aquaculture isolates to human pathogens, some assessment

of the risk of transfer of resistant organisms (or genes) to humans *via* food chain and the threats imposed by environmental contamination with antibiotic resistant bacteria.

The highly antibacterial producing *Enterococcus* strains isolated from both sea bream and sea bass which inhibit growth of pathogenic and spoiling bacteria should have a potential practical interest and offer a natural means for simultaneous application as probiotics and/or for preventing the development of *Listeria* in food stuffs.

6. References

Andrighetto, C.; Knijif E.; Lombardi, A.; Vancanneyt, M.; Kersters, K.; Swings, J. & Dellaglio F (2004). Phenotypic and genetic diversity of enterococci isolated from Italian cheeses. *Journal of Dairy Research*, Vol.68, pp.303-316

Angulo, F. J.; Nargund, V. N. & Chiller, T. C. (2004). Evidence of an Association Between Use of Anti-microbial Agents in Food Animals and Anti-microbial Resistance Among Bacteria Isolated from Humans and the Human Health Consequences of Such Resistance. *Journal of Veterinary Medecine*, Vol. 51, pp.374–379

Austin, B.; Stuckey, L.F.; Robertson, PAW.; Effendi, I. & Griffith, DRW. (1995). A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida,Vibrio anguillarum* and *Vibrio ordalii*. *Journal of Fish Diseases*, Vol.18, pp.93-96

Bairagi, A.; Sakar Ghosh; K., Sen; S.K. & Ray, A.K. (2002). Enzyme producing bacterial flora isolated from fish digestive tracts. *Aquaculture International* , Vol.10, pp.109–121

Bairagi, A.; Sarkar Ghosh, K.; Sen, S.K. & Ray, A.K. (2004). Evaluation of the nutritive value of Leucaena leucocephala leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and Bacillus circulans in formulated diets for rohu, Labeo rohita (Hamilton) fingerlings. *Aquaculture Research*, Vol. 35, pp. 436–446

Bakhrouf, A. ; Ben Ouada, H. & Oueslati, R. (1995). Essai de traitement des vibrioses du loup *Dicentrarchus labrax* dans une zone de pisciculture, à Monastir, Tunisie. *Marine Life*, Vol.5, pp. 47-54

Balcàzar, JL.; de Blas, I.; Ruiz-Zarzuela, I.; Cunningham, D., Vendrell, D. & Muuzquiz, JL. (2006). The role of probiotics in aquaculture. *Veterinary Microbiology*, Vol. 114, pp.173–186

Barton, M.D. (2000). Antibiotic use in animals feed and its impact on human health. *Nuritiont Research Reviews,* Vol.13, pp.79–299

Ben Kahla-Nakbi, A.; K., Chaieb; A., Besbes; T., Zmantar & A., Bakhrouf, (2006). Virulence and enterobacterial repetitive intergenic consensus PCR of *Vibrio alginolyticus* strains isolated from Tunisian cultured gilthead sea bream and sea bass outbreaks. *Veterinary Microbiology*, Vol.117, pp.321-327

Boggard van den, AE. & Stobberingh EE. (2000). Epidemiology of resistance to antibiotics-Links between animals and humans. *International Journal of Antimicrobial Agents,* Vol.***14***, pp.327-335

Bouamama, K. (2001). *Mytilus galloprovincialis* de la lagune de Bizerte : populations bactériennes et Biomarqueurs non spécifiques. Diplôme des études approfondies (DEA). Faculté des Sciences de Tunis, Institut des Sciences et Technologies de la Mer. Tunis, Tunisia

Boutiba, I.; Ghozzi, R.; Jouaihia, W.; Mahjoubi, F.; Thabet, L.. Smaoui, H.; Ben Hassen, A. Hàmmamí, A.; Kechrid, A. & Ben Redjeb, S. (2007). Résistance bactérienne aux antibiotiques en Tunisie : Données de 1999 A 2003, *Revue Tunisienne d'Infectiologie*, Vol. 1, pp. 5-11

Calo-Mata, P.; Arlindo, S.; Boehm. K. ; De Miguel, T. Pascoal A. & Barros-Velazquez, J. (2007). Current application and future trends of lactic acid bacteria and their bacteriocins for biopreservation of aquatic food products. *Food Bioprocess Technology*, Vol.1(1), pp.43-63

Celli, J. & Trieu-Cuot, P. (1998). Circularization of Tn916 is required for expression of the transposon-encoded transfer functions: characterization of long tetracycline-inducible transcripts reading through the attachment site. *Molecular Microbiology*, Vol. 28, pp.103–117

Chabbert Y.A (1982). L'antibiogramme. In : Bactériologie médicaleL. Le Miror, M. véron, (eds): Flammarion. Medecine Science. Paris, 205-212.

Chae-Woo, M.; Yun-Seok, C. & Kye-Heon, O. (2009). Removal of pathogenic bacteria and nitrogens by *Lactobacillus spp*. JK-8 and JK-11. *Aquaculture*, Vol.287, pp.266-270

Chahad Ouissal, B.; El Bour, M.; Mraouna, R.; Abdennaceur, H. & Boudabous, A. (2007). Preliminary selection study of potential probiotic bacteria from aquacultural areas in Tunisia. *Annals of Microbiology*, Vol.57 (2), pp.185-190

Chelossi, E. ; Vezzulli, L. ; Milano, A. ; Branzoni, M. ; Fabiano, M. ; Riccardi, G. & Banat, I.M., (2003). Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. *Aquaculture,* Vol. 219, pp.83–97

CIESM 2004. Novel contaminants and pathogens in coastal waters. CIESM Workshop Monograph N°26, Monaco "www.ciesm.org/online/monographs/Neuchatel.pdf"

Clewell, DB., Flannagan, SE. & Jaworsky. DD. (1995). Unconstrained bacterial promiscuity: the Tn*916*-Tn*1545* family of conjugative transposons. *Trends in Microbiology*, Vol.3, pp.229–236

Comité de l'Antibiogramme de la Société Française de Microbiologie, (1996). Statement 1996 CA-SFM. Zone sizes and MIC breakpoints for non-fastidious organisms. *Clinical Microbiology and Infecion,* Vol.2, Suppl. 1, pp.46-49

Dellali, M., (2001). Utilisation d'indicateurs microbiologiques et biochimiques chez *Ruditapes decussatus* et *Mytilus galloprovincialis* dans la biosurveillance de la lagune de Bizerte : Validation de certains biomarqueurs. Thèse de doctorat, Faculté des Sciences de Bizerte. Tunisia

El Bour, M.; Attia El Hilli, H.; Mraouna, R. & Ayari, W. (2001). Bacterial study of mesophilic Aeromonads distribution in shellfish. Proceeding of the fifth international conference on the Mediterranean coastal environment, *MEDCOAST*, pp.557- 565

Ennahar, S.; Aoude-Werner. D.; Assobhei, O. & Hasselmann, D. (1998). Antilisterial activity of enterocin 81, a bacteriocin produced by *Enterococcus faecium* WHE 81 isolated from cheese, *Journal of Applied Microbiology, Vol.*85, pp.521–526

Franz, CMAP; van Belkum, MJ.; Holzapfel, WH; Abriouel, H. & Galvez, A. (2007). Diversity of Enterococcal bacteriocins and their grouping in a new classification scheme. *FEMS Microbiology Review,* Vol.31, pp.293–310

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, Vol. 66, pp.365–378

Giraffa, G. (1995). Enterococcal bacteriocins: their potential as anti-*Listeria* factors in dairy technology. *Food Microbe,* Vol.12, pp.291–299

Gismondo, M.R.; Drago, L.; Lombardi, A. (1999). Review of probiotics available to modify gastrointestinal flora. *International Journal of Antimicrobial Agents,* Vol. 12, pp.287–292

Gram, L.; Melchiorsen, J.; Spanggaard, B.; Huber, I. & Nielsen, T.F. (1999). Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. *Applied and Environmental Microbiology, Vol.*65 (3), pp.969–973

Gullian, M.; Thompson, F. & Rodriguez, J. (2004). Selection of probiotic bacteria and study of their immunostimulatory effect in Penaeus vannamei. *Aquaculture,* Vol.233, pp.1–14

Huycke, MM.; Sahm, DF. & Gilmore, MS. (1998). Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. *Emerging Infectious Diseases*, Vol.4(2), pp.239-249

Irianto, A. & Austin, B. (2002a). Probiotics in aquaculture. *Journal of Fish Diseases*, Vol.25, pp.633–642

Irianto, A. & Austin, B. (2002b). Use of probiotics to control furunculosis in rainbow trout, Oncorhynchus mykiss (Walbaum). *Journal of Fish Diseases,* Vol.25, pp.333–342

Itami, T.; Asano, M.; Tokushige, K.; Kubono, K.; Nakagawa, A.; Noboru, T.; Nishimura, H.; Maeda, M.; Kondo, M. & Takahashi, Y. (1998). Enhancement of disease resistance of kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from Bifidobacterium thermophilum. *Aquaculture,* Vol.164, pp.277–288

Murray, BE. (1998). Diversity among multidrug-resistant enterococci. *Emerging Infectious Diseases, Vol.* 4, pp.37–47

Kato, T.; Matsuda, T.; Ogawa, E.; Ogawa, H.; Kato, H.; Doi, U. & Nakamura, R. (1994). Plantaricin-149, a bacteriocin produced by *Lactobacillus pluntarum* NRIC 149. *Journal of Fermentution and Bioengineering,* Vol.77, pp.277-282

Kuhn, I.; Iversen, A.; Burman, L.G.; Olsson-Liljequist, B.; Franklin, A.; Finn, M.; Aarestrup, F.; Seyfarth, A.M.; Blanch, A.R., Taylor, H.; Caplin, J.; Moreno, M.A.; Dominguez, L. & Mollby, R. (2000). Epidemiology and ecology of enterococci, with special reference to antibiotic resistant strains, in animals, humans and the environment. Example of an ongoing project within the European research programme. *International Journal of Antimicrobial Agents.* Vol.14, pp.337–342

Lategan, M.J. & Gibson, L.F. (2003). Antagonistic activity of *Aeromonas media* strain A199 against Saprolegnia sp., an opportunistic pathogen of the eel, *Anguilla australis* Richardson. *Journal of Fish Diseases,* Vol.26, pp.147–153

Lategan, M.J.; Torpy, F.R. & Gibson, L.F. (2004a). Biocontrol of saprolegniosis in silver perch Bidyanus bidyanus (Mitchell) by Aeromonas media strain A199. *Aquaculture,* Vol.235, pp.77–88

Lategan, M.J.; Torpy, F.R. & Gibson, L.F. (2004b). Control of saprolegniosis in the eel *Anguilla australis* Richardson, by *Aeromonas media* strain A199. *Aquaculture,* Vol.240, pp.19–27

Laukova, A.; M., Marekova & P. Javorsky. (1993). Detection and antimicrobial spectrum of a bacteriocin like substances produced by Enterococcus faecium CCM4231. *J. Letters in Applied Microbiology, Vol.*16, pp.257-260

Lukàsova, J. & Sustàckovà, A. (2003). Enterococci and Antibiotic Resistance: Review Article. *Acta. Veterinaria Brno*., Vol.72, pp.315-323

Marcinek, H.; Wirth, R.; Muscholl-Silberhorn, A. & Gauer, M. (1998). Enterococcus faecalis gene transfer under natural conditions in municipal sewage water treatment plants. *Applied and Environmental Microbiology,* Vol.64, pp.626-632

Mauguin, S. & Novel, G. (1994). Characterization of lactic acid bacteria isolated from seafood. *Journal of Applied Bacteriology,* Vol.76, pp.616–625

Marra, D. & Scott. JR. (1999). Regulation of excision of the conjugative transposon Tn916. *Molecular Microbio*logy, Vol.31, pp.609–621

Matyar, F. (2007). Distribution and antimicrobial multiresistance in Gram-negative bacteria isolated from Turkish sea bass (*Dicentrarchus labrax* L., 1781) farm. *Annals of Microbiology,* Vol.**57**, pp.35–38

Nogami, K. & Maeda, M. (1992). Bacteria as biocontrol agents for rearing larvae of the Crab *Portunus trituberculatus*. *Canadian Journal of Fish. and Aquatic Sciences*, pp.2373-2376

Ramirez, R.F. & Dixon, B.A. (2003). Enzyme production by obligate intestinal anaerobic bacteria isolated from Oscars (*Astronotus ocellatus*), angelfish (*Pterophyllum scalare*) and southern flounder (*Paralichthys lethostigma*). *Aquaculture* , Vol.227, pp.417–426

Rengpipat, S.; Phianphak, W.; Piyatiratitivorakul, S. & Menasveta, P. (1998). Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture* Vol.167, pp.301-313

Rengpipat, S., Rueangruklikhit, T. & Piyatiratitivorakul, S. (2008). Evaluations of lactic acid bacteria as probiotics for juvenile sea bass *Lates calcarifer*. *Aquaculture Research*, Vol.39, pp.134-143

Rezgui, I. (2010). Etude de l'antibioresistance chez deux espèces de poisons aquacoles en Tunisie: le loup (*Dicentrarchus labrax*) et la daurade (*Sparus aurata*). Master of Biology. University of Sciences of Tunis. Institut des Sciences et Technologies de la Mer. Tunis, Tunisia

Rice, E.W.; Messer, J.W.; Johnson, C.H. & Reasoner, D.J. (1995). Occurrence of high-level aminoglycoside resistance in environmental isolates of enterococci. *Applied and Environmental Microbiology*. Vol.61, pp.374–376

Rico-Mora, R.; Voltolina, D. & Villaescusa-Celaya, J.A. (1998). Biological control of Vibrio alginolyticus in Skeletonema costatum (Bacillariophyceae) cultures. *Aquacultural Engineering* Vol.19, pp.1–6

Rigos, G.; Nengas, I.; Alexis, M. & Troisi, G. (2004). Potential drug (oxytetracycline & oxolinic acid) pollution from Mediterranean sparid fish farms. *Aquatic Toxicology,* Vol.69, pp.281-288

Rigos, G. & Troisi, G. (2005). Antibacterial agents in Mediterranean finfish farming: a synopsis of drug pharmacokinetics in important euryhaline fish species and possible environmental implications. *Reviews in Fish Biology and Fisheries,* Vol.15, pp.53-73

Ringo, E. (1999). Lactic acid bacteria in fish: antibacterial effect against fish pathogens.In effects of Anti nutrients on the Nutritional Value of Legume Diets, Vol.8, ed.

Krogdahl, Mathiesen, SD. And Pryme, I. COST 98. 70-75, Luxembourg: EEC Publication.

Ringø, E. & Gatesoupe, F.-J. (1998). Lactic acid bacteria in fish: a review. *Aquaculture,* Vol.160, pp.177–203

Ringo, E. (2008). The ability of *Carnobacteria* isolated from fish intestine to inhibit growth of fish pathogenic bacteria: a screening study. *Aquaculture Research*, Vol.39, pp.171-180

Salminen, S.; Ouwehand, A.; Benno, Y. & Lee, Y.K. (1999). Probiotics: how should they be defined. *Trends in Food Science and Technology,* Vol.10, pp.107–110

Smith, P.; Hiney, M. & Samuelson, O. (1994). Bacterialresistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. *Annual Review of Fish Diseases,* Vol.4, pp.273–313

Smith, V.J.; Brown, J.H. & Hauton, C. (2003). Immunostimulation in crustaceans: does it really protect against infection?. *Fish and Shellfish Immunology,* Vol.15, pp.71–90

Sorum, H. (1998). Mobile drug resistance genes among fish bacteria. *APMIS Suppl.,* Vol.106, pp.74–76

Sorum, H. (1999). Antibiotic resistance in aquaculture. *Acta Veterinaria Scandinavica Suppl*., Vol.92, pp.29–36

Spanggaard, B.; Huber, I., Nielsen, J.; Sick. E.B.; Pipper, C.B.; Martinussen, T.; Slierendrecht, W.J. & Gram, L. (2001). The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. *Environmental Microbiology*, Vol.3(12), pp.755-765

Vaseeharan, B.; Lin, J. & Ramasamy, P. (2004). Effect of probiotics, antibiotic sensitivity, pathogenicity, and plasmid profiles of *Listonella anguillarum* like bacteria isolated from Penaeus monodon culture systems. *Aquaculture*, Vol.241, pp.77–91

Verschuere, L. ; Rombaut, G. ; Huys, G. ; Dhont, J. ; Sorgeloos, P. & Verstraete, W. (1999). Microbial control of the culture of Artemia juveniles through preemptive colonization by selected bacterial strains. *Applied and Environmental Microbiology*, Vol.65, pp.2527–2533

Verschuere, L.; Rombaut, G.; Sorgeloos, P. & Verstraete, W. (2000a). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Review,* Vol.64, pp.655–671

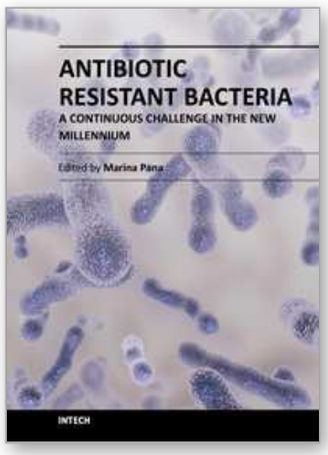
Verschuere, L.; Heang, H.; Criel, G.; Sorgeloos, P. & Verstraete,W. (2000b). Selected bacterial strains protect Artemia spp. from the pathogenic effects of Vibrio proteolyticus CW8T2. *Applied and Environmental Microbiology*, Vol.66(3), pp.1139–1146

Vijayabaskar, P. & Somasundaram, S. T. (2008). Isolation of bacteriocin producing lactic acid bacteria from fish gut and probiotic activity against common fresh water fish pathogen *Aeromonas hydrophila*. *Biotechnology*, Vol.7, pp.124-128

Vijayan, K.K.; Bright Singh, I.S.; Jayaprakash, N.S.; Alavandi, S.V.; Somnath Pai, S., Preetha, R.; Rajan, J.J.S. & Santiago, T.C. (2006). A brackish water isolate of *Pseudomonas* PS-102, a potential antagonistic bacterium against pathogenic vibrios in penaeid and non-penaeid rearing systems. *Aquaculture*, Vol.251, pp.192-200

Yan, L.; Boyd, K.G. & Burgess, J.G. (2002). Surface attachment induced production of antimicrobial compounds by marine epiphytic bacteria using modified roller bottle cultivation. *Marine Biotechnology,* Vol.4, pp.356–366

Zorrilla, I.; M., Chabrillón; S., Arijo; P., Díaz Rosales; E., Martínez-Manzanares; M.C., Balebona & M.A., Moriñigo, (2003). Bacteria recovered from diseased cultured gilthead sea bream (*Sparus aurata* L.) in southwestern Spain. *Aquaculture*, Vol.218, pp.11-20



Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium

Edited by Dr. Marina Pana

ISBN 978-953-51-0472-8
Hard cover, 576 pages
Publisher InTech
Published online 04, April, 2012
Published in print edition April, 2012

Antibiotic-resistant bacterial strains remain a major global threat, despite the prevention, diagnosis and antibiotherapy, which have improved considerably. In this thematic issue, the scientists present their results of accomplished studies, in order to provide an updated overview of scientific information and also, to exchange views on new strategies for interventions in antibiotic-resistant bacterial strains cases and outbreaks. As a consequence, the recently developed techniques in this field will contribute to a considerable progress in medical research.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ouissal Chahad Bourouni, Monia El Bour, Pilar Calo-Mata and Jorge Barros-Velàzquez (2012). Antimicrobial Resistance and Potential Probiotic Application of Enterococcus spp. in Sea Bass and Sea Bream Aquaculture, Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium, Dr. Marina Pana (Ed.), ISBN: 978-953-51-0472-8, InTech, Available from: http://www.intechopen.com/books/antibiotic-resistant-bacteria-a-continuous-challenge-in-the-new-millennium/antimicrobial-resistance-and-potential-probiotic-application-of-enterococcus-spp-in-sea-bass-and-sea

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen