Eastern Anatolian Journal of Science
Volume III, Issue I, 22-26
ISSN: 2149-6137

Antibiotic Resistance of Some Probiotic Strains in Aquaculture

DILEK ÖZCAN[1] and ŞÜKRÜ ÖNALAN[2]

[1]*Department of Biotechnology, Faculty of Agriculture, Yüzüncü Yıl University, Van, TURKEY*
[2]*Department of Fish Diseases, Faculty of Aquaculture, Yüzüncü Yıl University, Van, TURKEY*

Abstract

The term "probiotic" was firstly used to denominate microorganisms that have effects on other microorganisms. Antibiotics are used extensively because diseases are the most important factor limiting production and trade in aquaculture. Intensive use of antibiotics causes resistance to antibiotics, adverse effects on human health and many damages in the ecosystem.

In aquaculture, research on the use of probiotics to stabilize the bacterial populations in the water, reduce pathological bacterial load and improve water quality is increasing and the use of probiotics is widespread. Probiotics are single or mixed live microorganism cultures or metabolites there of which promote the development of the intestinal microflora of the host organisms, cause recovery and rapid growth in the oral cavity, gastrointestinal system, upper respiratory tract. On the other hand, probiotics help to detoxify potentially harmful compounds in foods and feed them by feeding them with amylases and proteases, digesting potentially inaccessible elements in the diet, stimulating the production of vitamins and the congestive immune system.
For these reasons it is important to identify antibiotic resistant strains and use them in aquaculture. In this study, 13 antibiotics (Kanamycin-K30, Penicillin-P10, Ampicillin-AM10, Erythromycin-E15, Clindamycin-DA2, Rifampin-RA5, Methicillin-ME5, Enrofloxacin-ENR5, Florfenicol-FFC30, Ciprofloxacin-CIP5, Nitrofurantoin-F300, Methicillin-ME5, Trimethoprim/ Sulphamethoxzole-SXT25) and 4 probiotic bacteria (*Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus paracasei*) were used.

As a consequence; the probiotic bacteria were determinated that *L. rhamnosus* was resistant to antibiotics of K30, P10, AM10, E15, DA2 and RA5; was intermediate T30 and then it was sensitive EN5, FFC30, CIP5, F300, ME5 and SXT25. *L. fermentum* was resistant to K30, AM10, DA2, T30, ME5 and RA5İ; was intermediate E15 and CIP5; and then it is sensitive to P10, ENR5, FFC30, F300 and SXT25. *L. paracasei* tested was resistant to K30, P10, AM10, E15, DA2 and RA5; was intermediate T30; and then it was sensitive to ENR5, FFC30, CIP5, F300, ME5 and SXT25. *L. casei* in probiotic bacteria used in the aquaculture was found resistant to antibiotics of K30, P10, AM10, E15, DA2, T30 and RA5 were sensitive, was intermediate ME5; it was sensitive to ENR5, FFC30, CIP5, F300 and SXT25.

Key words: Probiotics, Antibiotic resistance, Fish Diseases, Aquaculture.

Received: 30.11.2016
Revised: 20.03.2016
Accepted: 26.03.2017
Corresponding author: Dilek ÖZCAN, PhD
Yüzüncü Yıl University, Faculty of Agriculture,
Department of Biotechnology, Van, TURKEY
E-mail: dilekozcan@yyu.edu.tr
Cite this article as: D. Özcan and Ş. Önalan, Antibiotic Resistance of Some Probiotic Strains in Aquaculture, Eastern Anatolian Journal of Scienve, Vol. 3, Issue 1, 22-26, 2017.

Introduction

Aquaculture has become an important economic activity in many countries. In large-scale production facilities, where aquatic animals are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and result in serious economic losses. Prevention and control of diseases have led during recent decades to a substantial increase in the use of veterinary medicines (BALCA´ZAR, 2006).
Aquaculture is one of the fastest growing and food producing sectors. Aquaculture consists of all kinds of water animals and cultures of the plant in aquatic environments (PILLAY and KUTTY, 2005). Global production in the aquaculture sector reached 66.63 million tons in 2012 and aquaculture production in the year 2021 is projected to increase by 33% to reach 79 million tonnes (FAO, 2014). In the next decade, it is estimated that fish production will exceed the production of poultry, pork and beef. However, aquaculture is often damaged due to financial losses, especially fish diseases (FLEGEL, 2006).
In the field of aquaculture, extended the concept of

probiotic as "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" (QI, et al, 2009).

Aquatic animals are quite different from the land animals for which the probiotic concept was developed, and a preliminary question is the pertinence of probiotic applications to aquaculture (RINGØ, et al., 2005).

Man and terrestrial livestock undergo embryonic development within an amnion, whereas the larval forms of most fish and shellfish are released in the external environment at an early ontogenetic stage. These larvae are highly exposed to gastro- intestinal microbiota-associated disorders, because they start feeding even though the digestive tract is not yet fully developed and though the immune system is still incomplete. Thus, probiotic treatments are particularly desirable during the larval stages (GATESOUPE, 1999).

Gram-positive obligate or facultative anaerobes are dominant in the gastrointestinal microbiota of man and terrestrial farm animals. In human feces, the major bacterial groups are *Bacteroides*, Gram-positive anaerobic cocci, *Eubacterium*, and *Bifidobacterium.* whereas the predominant groups in pig feces are ‘‘streptococci’’ and ‘‘lactobacilli’. Most probionts belong to dominant or sub-dominant genera among these microbiota, e.g., *Bifidobacterium*, *Lactobacillus*, *Streptococcus.* Gram-negative facultative anaerobes prevail in the digestive tract of fish and shellfish, though symbiotic anaerobes may be dominant in the posterior intestine of some herbivorous tropical fish. *Vibrio* and *Pseudomonas* are the most common genera in crustaceans. marine fish and bivalves *Aeromonas*, *Plesiomonas* and Enterobacteriaceae are dominant in freshwater fish. A consequence of the specificity of aquatic microbiota is that the most efficient probiotics for aquaculture may be different from those of terrestrial species (CORDERO, et al., 2014).

The resident microbes benefit from a fairly constant habitat in the gastrointestinal tract of man and terrestrial livestock, whereas most microbes are transient in aquatic animals. These animals are poikilothermic, and their associated micro- biota may vary with temperature changes. Salinity changes may also influence microbiota and marine finfish are obliged to drink constantly to prevent water loss from the body. This continuous water flow increases the influence of the surrounding medium, in the same way as the water flow observed in filter-feeders, like bivalves, shrimp larvae and live food organisms. This influence is particularly important in larvae, when the gastric barrier is absent. Therefore, the intestinal microbiota of aquatic animals may change rapidly with the intrusion of microbes coming from water and food. In bivalves, the associated microbiota is very similar to those found in seawater and sediment. The same kinds of bacteria were found in the gut of *Penaeus japonicus* and in seawater, but normal members of microbiota may be introduced via the diet. In larval and juvenile fish, the influence of food has been clearly demonstrated. The influence of bacteria brought by live food organisms is particularly dramatic during first feding (CANNON, et al., 2005).

Materials and Methods

Bacteria strains

Four reference probiotic bacteria (*Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus paracasei*) used in this study were isolated at different times and used for study.

The probiotic bacteria used in the study were cultured on MRS (Man Ragosa and Sharpe) agar at 37 ^{o}C and fresh cultures were obtained (NIKOSKELAINEN at al., 2003).

Isolation of probiotic bacteria

The probiotic bacteria used and identified in this study were streaked on MRS agar and left for 24 hours incubation (NIKOSKELAINEN at al., 2003). After incubation, the contaminated white, white and opaque colonies formed in the petriol were removed and used for the MHA for antibiogram test (BALTA, et al., 2016).

Evaluation of results

The antibiotic discs (BIO) to be tested on the petri dish containing bacteria are placed in a sterile manner. The petri dishes were incubated at 37 degrees for 24 hours. After incubation, Zone diameters around the antibiotic discs were measured with a ruler in millimeters (Figure 1).

Figure 1. Measurement of antibiotic zone diameters

The measurement of antibiotic zone diameters were evaluated according to BALTA, et al., (2016) and the CLSI (*Clinical and Laboratory Standards Institute*), (CLSI, 2003), (Table 1).

Table 1. Limits for antimicrobial disk susceptibility test.

Antibiotic	Resistant	Intermediate	Susceptible
Kanamycin(K30)	≤22	23-25	≥26
Penicillin(P10)	≤14	—	≥15
Enrofloxacin(ENR5)	≤16	17-20	≥21
Florfenicol(FFC30)	≤14	15-18	≥19
Ampicillin(AM10)	≤13	14 – 16	≥17
Erythromycin(E15)	≤11	14 – 22	≥23
Ciprofloxacin(CIP5)	≤15	16-20	≥21
Clindamycin(DA2)	≤15	16 – 18	≥19
Nitrofurantoın(F300)	≤14	15 – 16	≥17
Oxytetracycline(T30)	≤15	16-25	≥26
Methicillin(ME5)	≤9	10-13	≥14
Rifampin(RA5)	≤16	17 – 19	≥20
Trimethoprim/Sulpha methoxzole(SXT25)	≤10	11 – 15	≥16

Findings

Antibiyogram test results

Antibiogram test results and images of probiotic bacteria used in this study against Kanamycin- K30, Penicillin- P10, Ampicillin- AM10, Erythromycin- E15, Clindamycin- DA2, Rifampin- RA5, Methicillin- ME5, Enrofloxacin- ENR5,Florfenicol-FFC30, CiprofloxacinCIP5, Nitrofurantoin-F300 and Trimethoprim/Sulpha methoxzole-SXT25 antibiotics are given below (Figure 2 and Table 2).

Figure 2. Images of antibiogram test results

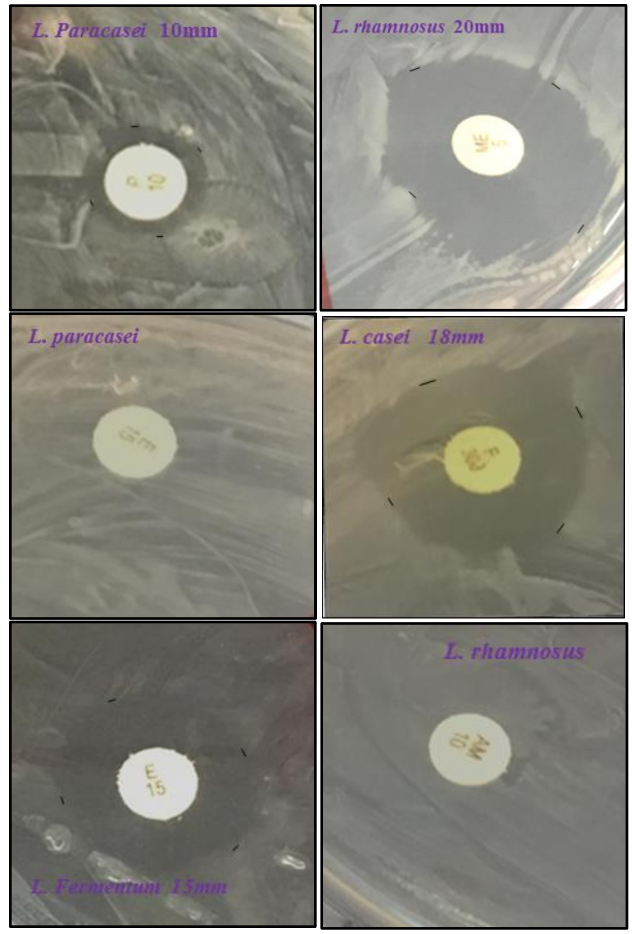

Table 2. Inhibitory zone diameter of probiotic bacteria species (mm)

Bacteria / Antibiyotic	*L. rhamnosus*	*L. casei*	*L. fermentum*	*L. paracasei*
Kanamycin	15	18,5	11	17
Penicillin	0	0	23	10
Enrofloxacin	26	25	22	28
Florfenicol	24	24	26	22
Ampicillin	0	8	8	0
Erythromycin	0	0	15	0
Ciprofloxacin	27	23	19	28
Clindamycin	0	0	8	0
Nitrofurantoın	19	18	25	20
Oxytetracycline	20	8	10	20
Methicillin	20	12	8	18
Rifampin	10	9	14	12
Trimethoprim/Sulphamethoxzole	29	27	23	29

Discussion and Results

A probiotic given at a site has more effect than the protective effect created by the host (BALCAZAR et al., 2006). Basically, the mechanisms and benefits of probiotics are: 1. The pathogen bacterium is out of competition. 2. Cultured organisms provide digestive enzymes, enzymatic contribution to digestion. 3. The probiotic bacteria can improve water quality by taking or decomposition organic or toxic substances in water. 4. It stimulates and enhances humoral and cellular immune response against pathogenic microorganisms. 5. It shows antiviral effect. 6. Allows the change of microbial metabolism by increasing or decreasing the levels of the relevant enzymes (PANİGRAHİ, A., and AZAD, I. S., 2007).

On the one hand It helps to reduce pathogenic bacteria in the environment by producing substances that prevent the growth and development of the pathogenic bacterium on the other hand competition with them for nutrients, oxygen and space (VASEEHARAN and RAMASAMY, 2003).

Table 3. Antibiotic resistance profiles for the Lactobacillus species

Bacteria / Antibiyotic	*L. rhamnosus*	*L. casei*	*L. fermentum*	*L. paracasei*
Kanamycin	S	R	S	S
Penicillin	S	R	R	S
Enrofloxacin	R	S	R	R
Florfenicol	R	S	R	R
Ampicillin	S	S	S	S
Erythromycin	S	S	I	S
Ciprofloxacin	R	R	I	R
Clindamycin	S	S	S	S
Nitrofurantoın	R	R	R	R
Oxytetracycline	I	S	S	I
Methicillin	R	I	S	R
Rifampin	S	S	S	S
Trimethoprim/ Sulphamethoxzole	R	R	R	R

S: Susceptible, I: Intermediate, R: Resistant

As a result; *L. rhamnosus* was resistant to antibiotics of Kanamycin (K30), Penicillin (P10), Ampicillin (AM10), Erythromycin (E15), Clindamycin (DA2) and Rifampin (RA5) was intermediate Oxytetracycline (T30) and then it was sensitive Enrofloxacin (ENR5), Florfenicol (FFC30), Ciprofloxacin (CIP5), Nitrofurantoın (F300), Methicillin (ME5) and Trimethoprim / Sulphamethoxzole (SXT25). Similarly (ROSSI et al. 2015) reported regarded high resistance to Oxytetracycline (intermediate), clindamycin, and erythromycin, and ampicillin in *L. rhamnosus.*

L. casei in probiotic bacteria used in the aquaculture was found resistant to antibiotics of Kanamycin (K30), Penicillin (P10), Ampicillin (AM10), Erythromycin (E15), Clindamycin (DA2), Oxytetracycline (T30) and Rifampin (RA5) were sensitive, was intermediate Methicillin (ME5); it was susceptible to Enrofloxacin (ENR5), Florfenicol (FFC30), Ciprofloxacin (CIP5), Nitrofurantoin (F300) and Trimethoprim/ Sulphamethoxzole (SXT25). SHAO et al., 2015 reported *L. casei* tested was resistant to Ampicillin, whereas sensitive to Ciprofloxacin, Erythromycin, Kanamycin.

L. fermentum was resistant to Kanamycin (K30), Ampicillin (AM10), Clindamycin (DA2), Oxytetracycline (T30), Methicillin (ME5) and Rifampin (RA5); was intermediate Erythromycin (E15) and Ciprofloxacin (CIP5); and then it is sensitive to Penicillin (P10), Enrofloxacin (ENR5), Florfenicol (FFC30), Nitrofurantoin (F300) and Trimethoprim/ Sulphamethoxzole (SXT25). MUTHUVELN (2011) stated that resistant to Amphicilin and Penicillin, and Enrofloxacine, and Kanamycin (SHARMA et al., 2014).

L. paracasei was resistant to Kanamycin (K30), Penicillin (P10), Ampicillin (AM10), Erythromycin (E15), Clindamycin (DA2) and Rifampin (RA5); was intermediate Oxytetracycline (T30); and then it was sensitive to Enrofloxacin (ENR5), Florfenicol (FFC30), Ciprofloxacin (CIP5), Nitrofurantoin (F300), Methicillin (ME5) and Trimethoprim/Sulphamethoxzole (SXT25). Reported resistance to ampicillin and clindamycin in *L. paracasei* Though the genetic determinants responsible for resistance could not be identified (ROSSİ et al. 2015).

Probiotics in aquaculture are promising for the future, but more works are needed in this regard. By means of these studies, the characteristics of the microorganisms in the intestinal tract should be determined and the mechanisms of action must be known in order to define the selection critic of possible probiotics. Furthermore, in vivo host/microorganism interactions, knowledge of microbial culture of probiotics as well as the natural macro flora functions and situations and microbial needs to be understood. In addition to these, studies on the effects of probiotics in practical and industrial use and probiotic use as a result of the addition of aquatic bacteria to aquatic environment are needed. (TURGUT at al., 2007).

Most importantly, the use of probiotics in practice in hatcheries and farms should also be evaluated economically, and the effect of production and cost/benefit analysis.

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