TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2010; 34(6): 501-506
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doi:10.3906/vet-0805-24

Dominant aerobic bacterial community of sea bass (*Dicentrarchus labrax* L.1758) larvae during weaning from Artemia to dry feed in culture conditions

Ayberk AYAZ, Süheyla KARATAŞ*

Fisheries Faculty, İstanbul University, Ordu Cad. No: 200 34470, Laleli, İstanbul - TURKEY

Received: 26.05.2008

Abstract: The aim of this study was to determine the dominant aerobic bacterial community of sea bass larvae during their weaning period in 2 different marine hatcheries in Muğla-Bodrum region. Samplings were made in January, March, and May 2005. Larvae and water samples were diluted in certain ratios and were inoculated to Marine Agar, TCBS Agar, Pseudomonas Agar, King Bee Agar, MRS agar, Blood Agar and Plate Count Agar, in that order. Water temperature, pH, saturation, salinity, and features of the system used were recorded.

Isolated bacterial strains were identified with standard biochemical methods and API 20E Rapid Identification Kits. In this study the total number of bacterial strains was detected as 109 and the number of all bacteria species was found as 18. At the end of this study Vibrio species were identified as the dominant species of the bacterial flora of sea bass larvae.

Key words: Dominant bacterial flora, *Dicentrarchus labrax*, Artemia, Vibrio

Introduction

There were significant developments reported on the aquaculture sector in Turkey in recent years, similar to other agricultural activities. Marine fish harvest was 35 t in 1986, but increased up to 25,239 t in 1999, and reached 69,673 t in 2005 (1,2). Successfully reared fish species in Turkey are sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), and rainbow trout (*Oncorhynchus mykiss*) (3).

One of the important elements affecting the aquaculture is fish diseases. The mortality caused by diseases in larval stages makes up approximately 50% of economical losses. The pathogenic and opportunistic bacteria are the cause of high mortalities at the first feeding stages of fish larvae (4). At these stages, the fish larvae, which get easily accustomed to live on artificial feed, may show different characteristics in their bacterial microflora. The kind of non-infectious bacteria, found in the normal flora of fish, can cause diseases if optimum conditions are not provided (5-8)

During the cultivation of marine fish, along with the hatching of eggs, interactions occur between biological surfaces and bacteria. These interactions can be regarded as the sign of native intestinal flora as well as an infectious disease. Bacteria can exist abundantly in water and they may easily roam between the host and its habitat because water conditions may provide an ideal medium, with nutrients and lots of organic material, for some bacterial growth (7,9,10)

* E-mail: skaratas@istanbul.edu.tr

The intestinal bacterial flora of fish is very important, because the bacterial flora has a continuous and dynamic effect on the host's immune system. Bacteria are a key in promoting the early development of the mucosal immune system both in terms of its physical components and function and continue to play a role later in life. Bacteria stimulate the lymphoid tissue associated with the gut mucosa to produce antibodies against pathogens. The immune system recognizes and fights against harmful bacteria. On the other hand, some microbial floras of the fish and water function as probiotics. Taking into account all factors mentioned above, the aim of this study was to determine the dominant aerobic bacterial intestinal flora of sea bass larvae during their weaning period in 2 different marine hatcheries.

Materials and methods

Sampling was carried out in January, March, and May 2005 in 2 hatcheries of Bodrum Peninsula (Turkey), located in open or semi-closed water circuit systems. At each site, 3 samplings were made. All sampled sea bass larvae were at their weaning stage and approximately 30-50 days old. The samples were taken while larvae were fed with Artemia + dry feed.

In this study, methods of Muroga et al. (11) and Grisez et al. (12) were applied and 2 tanks were selected for each hatchery. Before sampling, 3 different points for each tank were selected and 7-8 sea bass larvae were collected from each of those points. One water sample was also taken from each tank. The larvae sampled via sterile Pasteur pipettes were put in sterilized standard glass laboratory tubes and transferred to the laboratory in a cold storage box to be examined. The larvae were disinfected by 10% benzal conium chloride solution after the sea water was completely removed. After the disinfection, larvae were washed 5 times with PBS solution and all Pasteur pipettes, which were used in sampling, were fully sterilized. Disinfected and washed larvae were put into the tubes containing 2 mL PBS each. The larvae were homogenised manually. The homogenate was serially diluted in the ratios of 1/100, 1/10,000, and 1/100,000.

Of the diluted samples, 100 µL was inoculated to Marine Agar, Thiosulfate Citrate Bile Salt Agar (TCBS), Pseudomonas Agar, King Bee Agar, MRS agar, Blood Agar, and Plate Count Agar. Every plate was incubated at 23-25 °C for 3-5 days. After the incubation, the colonies were counted and classified (representing different morphologies, in each plate colony). They were picked from different dilution plates of a sample. Gram Staining, Motility, Cytochrome Oxidase, Catalase, O/F, O/129, and API 20E were tested for the identification of the bacteria.

The rapid kits were applied according to the recommendations of BioMerieux, the producer company. Colonies were diluted in sterile pure water and API strips were inoculated according to the manufacturer's instructions (BioMerieux, France). Strips were read after 24-72 h length incubations at 23-25 °C. The results were evaluated using API Lab Plus software. Undefined strains were also evaluated according to the tables created by Buller (13).

Results

In this study, samplings in 2 different marine fish hatcheries in Bodrum peninsula were carried out. At the end of the study, the dominant aerobic intestinal bacterial flora of sea bass larvae was determined.

The characteristics of 2 hatcheries at the time of sampling are presented in the Table.

We have isolated and identified *Aeromonas hydrophila*, *Vibrio cholerae*, *V. alginolyticus*, *Vibrio* sp., *V. splendidus*, *V. proteolyticus*, *V. pelagius*, *Flavobacterium* sp., *Acinetobacter/Moraxella* sp., *Pseudomonas* sp., *P. anguilliseptica*, *P. aeruginosa*, *Moritella marina*, *V. fluvialis*, *V. vulnificus*, *V. tubiashii*, *V. harveyi*, *V. diaboliticus* as a result of the 3 sampling periods. The number of the final total bacterial strain was 109; during this study, the number of the final identified species was 18 (Figure 1). The isolation percentages of the dominant bacterial species identified from hatchery 1 and hatchery 2 are represented in Figure 2. The numbers of *Vibrio* strains isolated and identified (water and larvae) from hatchery 1 and hatchery 2 are given in Figure 3.

Table. Characteristics of the 2 hatcheries at the time of sampling.

	Hatchery 1			Hatchery 2		
	Sampling 1	**Sampling 2**	**Sampling 3**	**Sampling 1**	**Sampling 2**	**Sampling 3**
Period	Artemia + Dry feed	Artemia + Dry feed	Artemia+ Dry feed	Artemia + Dry feed	Artemia + Dry feed	Artemia + Dry feed
Age (days)	50 days	50 days	49 days	47 days	47 days	34 days
Water temperature	19 °C	19 °C	20.2 °C	18.7 °C	18.7 °C	18.3 °C
Salinity	34 ‰	34 ‰	38 ‰	40 ‰	40 ‰	35 ‰
Oxygen	9.2	9.2	9.3	6.2	6.2	9
pH	7.4	7.4	7.4	7.3	7.3	7.8
Tank capacity	16 t	16 t	16 t	18 t	18 t	18 t
Diet size	150-200 µ	150-200 µ	200-400 µ	200-300 µ	200-300 µ	200-300 µ
System	Open system	Open system	Open system	Semi-closed system	Semi-closed system	Semi-closed system
Water	well water	well water	well water	sea water	sea water	sea water

Discussion and conclusion

In this study, at the end of 3 sampling periods in both hatcheries, the species *Aeromonas hydrophila*, *Vibrio cholerae*, *V. alginolyticus*, *Vibrio* sp., *V. splendidus*, *V. proteolyticus*, *V. pelagius*, *Flavobacterium* sp., *Acinetobacter/Moraxella* sp., *Pseudomonas* sp., *P. anguilliseptica*, *P. aeruginosa*, *Moritella marina*, *V. fluvialis*, *V. vulnificus*, *V. tubiashii*, *V. harveyi*, and *V. diaboliticus* were identified.

Several researchers reported that *Vibrionaceae* are the most common members of the gut flora of feeding larvae (10-12,14). Grisez et al. (12) reported that the incidence of non-vibrio taxa during the first weeks of larval development was as high as the incidence of vibrios because the digestive tract and the secretion of gastric juices in larval marine fish was not yet sufficiently developed to produce selective conditions. Gastric selection is detected after the larvae reached the age of 30 days (12). In accordance with their results, our findings showed that the bacterial flora of sea bass larvae contains a wide variety of bacterial species with the dominant species being the members of Vibrio genus. This result is not surprising because the age of the larvae was more than 30 days (30-50 days) when we took the samples.

We observed that the incidence of *Vibrio* species was high in sea bass larvae in both hatchery 1 (fish and water) and hatchery 2 (fish and water) (more than 60%). *Acinetobacter/Moraxella* sp., *Flavobacterium* sp. and *V. alginolyticus* were isolated during all sampling periods as well.

Muroga et al. (11) have isolated 47% *Vibrio anguillarum*, *Vibrio alginolyticus,* and *Vibrio vulnificus* as well as 30% *Pseudomonas* sp., in the bacterial intestinal flora of black sea bream and red sea bream. Scientists have reported that the normal intestinal flora can change depending upon the feed and water

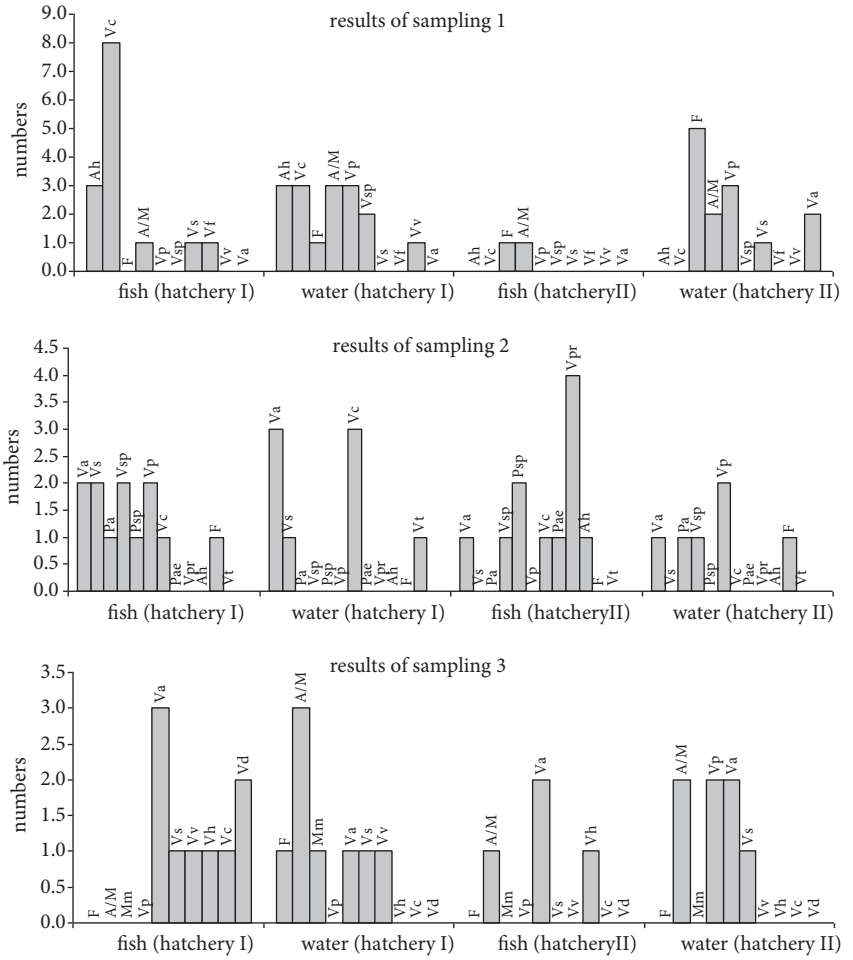

Figure 1. Figure 1. Bacterial strains identified and isolated (water and larvae) from hatchery 1 and hatchery 2 (Ah: *Aeromonas hydrophila*, Vc:*Vibrio cholerae*, Va: *V. alginolyticus*, Vsp.: *Vibrio* sp., Vs.:*V. splendidus*, Vpr: *V. proteolyticus*, Vp: *V. pelagius*, F: *Flavobacterium* sp, A/M: *Acinetobacter/Moraxella* sp., , Psp: *Pseudomonas* sp., Pa: *P. anguilliseptica*, Pae; *P. aeruginosa*, Mm: *Moritella marina*, Vf: *V. fluvialis*, Vv:*V. vulnificus*, Vt: *V. tubiashii*, Vh: *V. harveyi*, Vd: *V. diaboliticus*).

(7,11,12). According to the results of Grisez et al. (12), *Vibrio anguillarum* is dominant in fish that are fed with rotifers and *Vibrio alginolyticus* is dominant in fish that are fed with Artemia. We identified *Vibrio cholerae non O1* as dominant especially in hatchery 1 with larvae fed Artemia + dry feed. However, *Vibrio anguillarum* was not dominant in the gut and water. We identified more *Vibrio* species in hatchery 1 than in hatchery 2. The total number of bacteria isolated from hatchery 1 (water and fish) was also higher than hatchery 2 (water and fish).

Sugita et al. (15) studied the antibacterial abilities of intestinal microflora of Japanese flounder (*Paralichthys olivaceus*) larvae and juveniles against bacterial pathogens. They found substantial numbers of *Vibrio* sp. and *Acinetobacter*/*Moraxella* sp. on their samples of rotifer, Artemia, and Japanese flounder. Similarly, in this study, *Vibrio* sp. and *Acinetobacter*/*Moraxella* sp. were isolated from the water as well as from the sea bass larvae.

Furthermore, we identified *Moritella marina,* which was not the case in the studies of Newman et

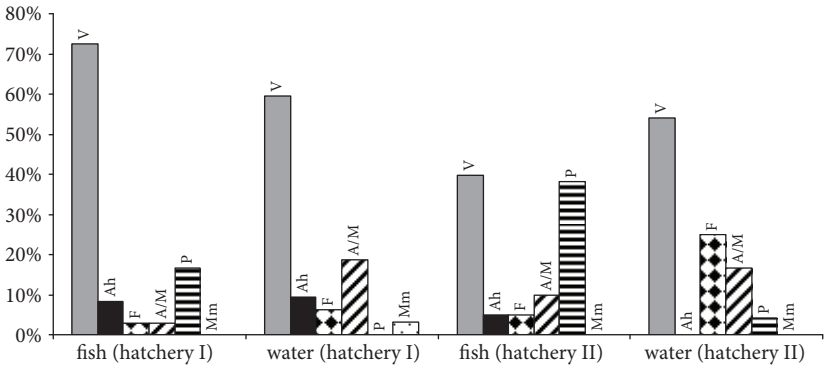

Figure 2. The percentages of bacterial strains identified and isolated (water and larvae) from hatchery 1 and hatchery 2. Ah: *Aeromonas hydrophila*, V:*Vibrio* strains, F: *Flavobacterium* sp., A/M: *Acinetobacter/Moraxella* sp., P: *Pseudomonas* sp., Mm: *Moritella marina.*

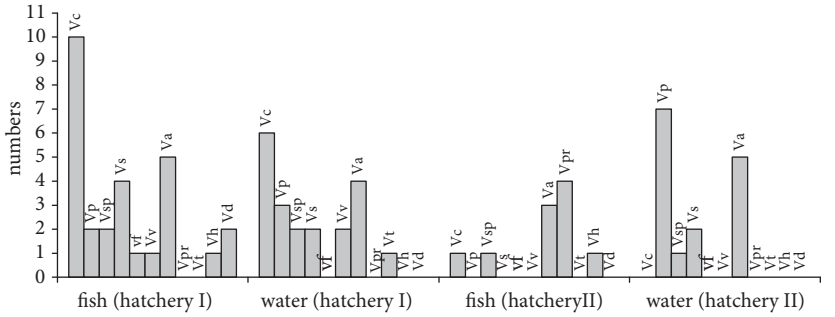

Figure 3. The numbers of *Vibrio* strains identified and isolated (water and larvae) from hatchery 1 and hatchery 2. (Vc:*Vibrio cholerae*, Vp: *V. pelagius*, Vsp.: *Vibrio* sp., Vs:*V. splendidus*, Vf: *V. fluvialis*, Vv:*V. vulnificus*, Va: *V. alginolyticus*, Vpr: *V. proteolyticus*, Vt: *V. tubiashii*, Vh: *V. harveyi*, Vd: *V. diaboliticus*).

al. (16), Muroga et al. (11), Toranzo et al. (17), Grisez et al. (12), Blanch et al. (14), Nedoluha and Westhoff (18), and Sugita et al. (15).

In marine hatcheries, it is known that the relationship between larvae and bacteria starts with the hatching period (7). Until now, there are not many studies available about the bacterial flora of sea bass larvae. There is still no clear information about the interactions between specific bacteria and the host larvae.

Based on the findings of the present study, we can say that Vibrio species could have an important role for sea bass larvae in hatchery systems. The bacterial intestinal flora of fish, which always changes depending on several factors, such as environmental variability, feed, and stress, is not known well. Especially a better understanding of the potential opportunistic pathogens would be helpful in future studies concentrating on the antagonistic interactions between healthy bacteria and opportunistic pathogens in sea bass larvae.

Acknowledgements

This present work was supported by The Research Fund of İstanbul University, Project Numbers: 270/23082004 and T-551/21102004.

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