

## Mediterranean Marine Science

Vol 17, No 1 (2016)

VOL 17, No 1 (2016)



### Characterization of *Micrococcus luteus* and *Bacillus marisflavi* Recovered from Common Dentex (*Dentex dentex*) Larviculture System

T. AKAYLI, G. ALBAYRAK, Ç. ÜRKÜ, Ö. ÇANAK, E. YÖRÜK

doi: [10.12681/mms.1322](https://doi.org/10.12681/mms.1322)

#### To cite this article:

AKAYLI, T., ALBAYRAK, G., ÜRKÜ, Ç., ÇANAK, Ö., & YÖRÜK, E. (2015). Characterization of *Micrococcus luteus* and *Bacillus marisflavi* Recovered from Common Dentex (*Dentex dentex*) Larviculture System. *Mediterranean Marine Science*, 17(1), 163–169. <https://doi.org/10.12681/mms.1322>

## Characterization of *Micrococcus luteus* and *Bacillus marisflavi* Recovered from Common Dentex (*Dentex dentex*) Larviculture System

T. AKAYLI<sup>1</sup>, G. ALBAYRAK<sup>2</sup>, Ç. ÜRKÜ<sup>1</sup>, Ö. ÇANAK<sup>1</sup> and E. YÖRÜK<sup>2</sup>

<sup>1</sup>Department of Fish Disease, Faculty of Aquatic Sciences, University of Istanbul, 34470, Ordu Cad. No:200, Laleli-Istanbul/Turkey

<sup>2</sup>Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, 34134, Istanbul, Turkey

Corresponding author: [takayli@yahoo.com](mailto:takayli@yahoo.com)

Handling Editor: Adriana Zingone

Received: 31 March 2015; Accepted: 11 September 2015; Published on line: 15 February 2016

### Abstract

In this manuscript, thirty yellow-pigmented Gram-positive bacteria were isolated from natural intestine microflora and from sea water around the marine cage of a rearing tank of common dentex (*Dentex dentex*), in the Aegean Sea on the Turkish coast and were characterized. Eighteen isolates were assigned to the species *Micrococcus luteus*, the other twelve to the species *Bacillus marisflavi*. Eight representative strains, six from *B. marisflavi* and two from *M. luteus*, were chosen for further 16S rDNA analyses. A pathogenicity assay for the isolated bacterial strains was carried out in rainbow trout and it evidenced absence of pathogenicity in the tested strains. The isolated strains were tested for in vitro antagonistic activity against *Listonella anguillarum*, a pathogen bacterium diffused in Mediterranean aquaculture and affecting various fish species. The isolated bacterial strains showed antagonistic activity against the pathogenic bacterium, suggesting a possible role of isolates as probiotics. In this study, for the first time, bacterial strains of the species *B. marisflavi*, known as an environmental species, were recovered in the gut microbiota of a healthy fish. The use of the isolates characterized in this study, mainly the yellow-pigmented bacterium, is suggested as possible probiotics to improve fish health, along with alternative methods of maintaining a healthy environment.

**Keywords:** *Micrococcus luteus*, *Bacillus marisflavi*, Bacterial characterization, *Dentex dentex*.

### Introduction

Common dentex (*Dentex dentex*) is a sparid fish species which has been cultured in Mediterranean countries, including Turkey, since the early 2000's (Abellan, 1999; Firat *et al.* 2003). Aquaculture of this species has greatly developed due to the mass scientific studies on the morphology, physiology and aquaculture of this fish (Efthimiou *et al.*, 1994; Firat *et al.*, 2003; Koumoundouros *et al.*, 2004). The main obstacle to further development are the disease problems, which are especially observed in the larval stages of this fish in culture systems (Rueda & Martinez, 2001).

Fish gut flora generally consists of a community of aerobic, facultative anaerobic and obligate anaerobic bacteria which are also present in the rearing system (Udey, 1978; Trust *et al.*, 1979). The increase in knowledge on the gut flora of cultured fish larvae allows to improve the detection of the potential source of pathogenic bacteria and eventually control disease outbursts, thus preventing economic losses, and it also contributes to a more efficient use of probiotics (Gomez-Gil *et al.*, 2000; Ganguly & Mukhopadhyay, 2010). Despite the fact that there are many studies on the gut flora of the larval stages of other sparids such as the gilt-head sea bream and sea bass (Grisez *et al.*, 1997; Savas *et al.*, 2005), there are insufficient

data on the gut flora of the common dentex. In a previous study, we determined the larval and juvenile gut flora of common dentex cultured in Turkey (Akayli *et al.*, 2015). In that study, we recovered some yellow pigmented Gram-positive bacteria but we could identify them only at the genus level using conventional biochemical tests.

16S rRNA gene sequencing plays an important role in accurately identifying species in microbial communities (Woo *et al.*, 2009) together with biochemical profiles (Al-sina & Blanch, 1994). This gene consists of conserved and varied nucleotide sequences used for determination based on sequencing approaches (Bintang *et al.*, 2014). Since the function of this gene has not changed over time, conserved gene sequence differences can be confidently used for bacterial definition at the species level (Janda & Abbott, 2007).

Due to the negative economical results of fish diseases, one of the main study area in aquaculture is their prevention by using consumer- and environment-friendly economical methods. Various bacterial groups present in fish digestive tract and their environment are beneficial to fish health (Gatesoupe, 1999; Gomez-Gil *et al.*, 2000; Spanggaard *et al.*, 2001) because they inhibit the colonization of potential pathogens due to their antagonistic affect (Verschuere *et al.*, 2000; Irianto & Austin, 2002). *Bacillus* and *Micrococcus* species are among a wide

range of Gram-positive bacteria (*Carnobacterium*, *Enterococcus*, *Lactococcus*, *Lactobacillus* and *Streptococcus*) which have been evaluated as probiotics in aquaculture with successful results (Irianto & Austin, 2002).

Most importantly, antagonistic activity has been detected in the members of the genus *Bacillus* (Bernan *et al.*, 1997; Ganguly & Mukhopadhyay, 2010; Austin & Austin, 2012). Carotenoids are both necessary and sufficient to promote bacterial pathogenicity. In many cases, the microbial pigment contributes to disease pathogenesis and directly promotes immune suppression by interfering with host immune clearance mechanisms or exerting pro-inflammatory or cytotoxic properties (Liu *et al.*, 2005; Khaneja *et al.*, 2010). Some studies have been conducted on the probiotic use of pigmented Gram-positive bacteria against different fish pathogens (Lemos *et al.*, 1985; Nair & Simidu, 1987), but their antagonistic effect has not been investigated against *Listonella anguillarum*.

Alternative methods of maintaining healthy environments for aquacultured fish have been investigated (Koumoundouros *et al.*, 2004). Particularly, *L. anguillarum* is a common problem in Mediterranean aquaculture and affects many fish species. However, this organism developed antibiotic resistance and hence treatment of the disease became more complicated. The use of probiotics in aquaculture is becoming increasingly important to improve growth or survival of farmed aquatic species and provides protection from diseases (Gatesoupe, 1999; Gomez-Gil *et al.*, 2000).

The main aim of this study is the biochemical and molecular characterization of yellow-pigmented bacteria recovered from the rearing tank and indigenous gut microbiota of common dentex (*Dentex dentex*). Other purposes of this study are the investigation of the pathogenicity of these bacteria in rainbow trout and determination of their *in vitro* antagonistic effect against *Listonella anguillarum*.

## Materials and Methods

### Bacterial isolation and identification

Five sampling studies were done between 2009 and 2010 in a commercial land-based hatchery located in the Aegean Sea on the Turkish coast. Fish samples were examined aseptically, dissected under sterile conditions and bacterial inoculations were made from the rearing water and gut samples of common dentex larvae from different exogenous feeding stages (non-feeding, rotifer, *Artemia* spp. and artificial pellet feeds, respectively) (Muroga *et al.*, 1987). Briefly, water and gut samples were diluted at different proportions (1/10, 1/100, 1/1000 and 1/10000) with sterile phosphate buffer saline (PBS) which was prepared by using commercial PBS tablets (Medicago AB, Sweden) with a final pH 7.4 and spread onto various media (Marine Agar 2216 - MA [Difco], Plate Count Agar – PCA

[Acumedia] and Tryptic Soy Agar – TSA [HiMedia]). An extra 1.5 % NaCl was added to commercial formulation of PCA and TSA. After incubation at 22 °C for 2-5 days, bacterial colonies were grouped depending on their color, shape, margins and consistency differences. Especially yellow pigmented colonies were selected and standard morphological and biochemical methods such as Gram-staining, hanged drop motility test, oxidase and catalase activities etc. and API STAPH system was used for further identification of these strains. Gram-stained preparations were examined under light microscope for the determination of Gram characteristics and shape of the bacteria.

### 16S rRNA gene sequencing

A partial region of the 16S rRNA gene was amplified from genomic DNAs (extracted by using a commercial kit; Thermo-K0721, USA) with universal primer pair (27F: 5'-AGAGTTTGATCTGGCTCAG-3' and 1492R: 5'-ACCTTGTTACGACTT-3') developed by Lane (1991). PCR was performed using modified conditions and cycling profile reported by Eder *et al.* (1999). After purification, amplicons were sequenced based on a chain termination method (kit: Applied biosystems, USA) with ABI PRISM 3100. Chromatograms were monitored and analysed by Chromas Pro 1.7.6 (Technelysium, Australia). Two directional nucleotide sequence data were assembled with DNA Dragon software (1.1.9.1). Similarity was searched with BLASTN through the NCBI (Altschul *et al.*, 1997). 16S rDNA sequences were subjected to CLUSTALW analysis using MEGA 6.0 (Tamura *et al.*, 2013). A similarity matrix was constructed with the neighbor-joining algorithm of Jones-Thornton-Taylor model (Tamura & Nei, 1993). A dendrogram was generated according to the cluster analysis using the UPGMA. 16S rDNA sequence data were deposited in Genbank by using Sequin 13.05 (Benson *et al.*, 2000).

### Pathogenicity assay

For the determination of the pathogenicity of isolated strains belonging to *B. marisflavi* and *M. luteus*, 3 experimental groups and a control group were created with 200 rainbow trout (mean weight 5-7 g) for each bacterial species. Bacterial suspensions of 10<sup>6</sup>, 10<sup>7</sup> and 10<sup>8</sup> cells/ml were prepared with PBS (phosphate buffered saline) solution for each bacterial species and fish groups were immersed in these bacterial suspensions. Later, fish groups were reared for 30 days and were monitored for possible disease symptoms and mortalities for the determination of pathogenicity.

### Antagonistic activity assays

After identification of the bacteria, yellow pigmented Gram-positive isolates were screened for antagonistic effect. *In vitro* antagonistic effect of these bacteria were determined with the Kirby-Bauer disc diffusion method

modified by Bhunia *et al.* (1988) on five different *Listonella anguillarum* strains that were recovered from the internal organs of diseased marine cultured gilt-head sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and fresh-water cultured rainbow trout (*Oncorhynchus mykiss*) sampled from Turkey. Fresh cultures of *L. anguillarum* strains were streaked onto Muller-Hinton agar and paper discs that were dipped into separate mixtures of *B. marisflavi* and *M. luteus* strains were placed on the agar surface. This assay was repeated three times for both bacterial species. An erythromycin disc was used as a positive control. Clear zones around discs were evaluated as positive results and their diameters were measured after incubation.

## Results

In this study, a total of 30 yellow-pigmented Gram-positive isolates were recovered and identified based on their biochemical characteristics and 16S rRNA gene sequences. Eighteen were Gram-positive, non-motile, cocci-shaped tetrads, oxidase and catalase positive isolates, which were identified as *Micrococcus luteus*. Twelve were Gram-positive, motile, facultative anaerobe, gas-forming from glucose, catalase negative, oxidase-positive, spore-forming bacilli-shaped isolates, which were identified as *Bacillus marisflavi* (Table 1). These two bacterial species were especially recovered from the natural intestinal microflora of non-feeding larvae and other following larval stages. Also *M. luteus* was recovered from sea water around the marine cages.

Of the 30 yellow pigmented Gram-positive bacteria, six *B. marisflavi* (AKAYLI 09-14) and two *M. luteus* (AKAYLI 15 and 122) yielded a band of 1.5 kb, corresponding to a partial 16S rDNA region. After assembling analysis, crude nucleotide data obtained from chromatograms were ranged from 1.3 to 1.4 kb. All *B. marisflavi* strains showed significant nucleotide sequence homologies (except value < 0.05 and bit scores > 50) with the reference 16S rDNA sequence (accession number KC414706.1) of *B. marisflavi* (Table 2). Similarly, two *M. luteus* strains showed high levels of similarity (except value < 0.05 and bit scores > 50) with the reference sequence (KF733697.1) of *M. luteus* (Table 2). When compared to each other and their own reference species via CLUSTALW, similarity among bacteria ranged from 69.67 to 100%. The highest similarity percentage was detected among three *B. marisflavi* strains (AKAYLI 09, 10 and 13), and between one of them and the reference sequence; besides and interestingly, 98% similarity between *B. aquamaris* (NR\_025241.1) and AKAYLI 09 was detected. The most genetically distant strains (69.67%) were determined as AKAYLI 11 and AKAYLI 15, belonging to *B. marisflavi* and *M. luteus*, respectively (Table 3). Deletions, insertions and SNPs in

**Table 1.** General phenotypic and biochemical characteristics of the yellow pigmented Gram-positive bacter.

Characteristics	<i>Bacillus marisflavi</i>	<i>Micrococcus luteus</i>
Gram staining	+	+
Catalase	+	+
Oxidase	-	+
Motility by flagella	+	-
Gliding motility	-	-
O/F	O	O
VP	+	-
Indole	-	-
Arginine	V	V
Ornithine	-	-
Lysine	-	-
MR	+	-
β-galactosidase	+	-
Acid production from		
D-glucose	+	-
L-arabinose	-	-
D-xylose	-	-
D-mannitole	-	V
Degradation of		
Casein	+	-
Gelatin	+	+
Starch	+	ND
Aesculin	-	+
Utilization of citrate	+	-
Urease	V	V
Nitrate reduction	+	-
Growth in		
2% NaCl	+	+
5% NaCl	+	+
7% NaCl	-	+

+: positive -: negative V: variable ND: not detected O: oxidative

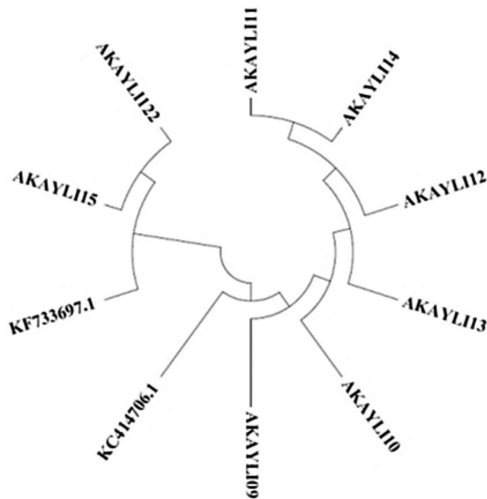
16S rDNA were detected as distinguishing alterations for the two species (Fig. 1). Nucleotide data of these 8 strains were deposited under Genbank with accession numbers KJ541103, KJ560871, KJ560870, KJ560872, KJ560874, KJ560873, KM062059 and KM062060 (Table 2). An UPGMA dendrogram displayed monophyletic branching (Fig. 2). Gram-positive bacteria found in probiotic communities consist of two groups; group I and group II. While six *B. marisflavi* strains were grouped together with their own reference genome KC414706.1 in group I, two *M. luteus* were clustered in group II with the reference strain KF733697.1.

Bacterial strains of *B. marisflavi* and *M. luteus* were determined as non-pathogenic in rainbow trout because they did not cause any important clinical symptoms nor mortality during 30 days investigation after the bacterial challenge.

As a result of the antagonistic activity assays, it was determined that both *B. marisflavi* and *M. luteus* strains isolated in this study showed *in vitro* antagonistic activity against *L. anguillarum* and produced inhibition zones of various diameters (5–30 mm) around the paper discs.







**Fig. 2:** Dendrogram, constructed by UPGMA analysis, including 8 bacterial strains together with their references

However, *B. marisflavi* showed a greater inhibitory activity against *L. anguillarum* (Table 4) than *M. luteus* isolates in general.

## Discussion

Because of high mortality rate during the larval stages (Rueda & Martinez, 2001), alternative methods need to be developed to maintain a healthy microbial environment in the larval rearing tanks (Koumoundouros *et al.*, 2004). Identification of bacterial communities present in the fish gut microbiota and the rearing environment can provide useful information for the improvement of the success of the aquaculture operations and fish welfare (Gatesoupe, 1999; Gomez-Gil *et al.*, 2000; Spanggaard *et al.*, 2001). Here we report the identification and characterization of yellow-pigmented Gram-positive bacteria that are commonly and abundantly recovered from the gut microbiota and the rearing environment of cultured common dentex in Turkey.

In this study, 30 yellow pigmented Gram-positive bacterial isolates were recovered from the intestine of common dentex larvae and tank water. Biochemical tests showed that 60% of these yellow pigmented bacteria were *M. luteus* and 40% were *B. marisflavi*. Yoon *et al.* (2003) compared rDNA sequences of one *B. marisflavi* and one *B. aquamaris* strains to that of other *Bacillus* species. They detected similarity of less than 97% and reported that these two strains belonged to different species. Wieser *et al.* (2002) characterized nine yellow pigmented bacterial strains and analysed their 16S rRNA gene sequences. They reported that all isolates belonged to *M. luteus* and that the minimum homology value among them and the *M. luteus* reference (DSM20030T) was 97.5%. Despite the hypothesis that sequence similarity within the same species can be minimum 97.5% (Stackebrandt & Goebel, 1994), according to recent limited data, 98% similarity between *B. aquamaris* (NR\_025241.1) and

AKAYLI 09 was detected. As the information in the gene sequencing database increased, distinction between *B. marisflavi* and *B. aquamaris* would be enlightened more accurately and a lower similarity between these two species can be detected.

*Micrococcus luteus* is a natural yellow-pigmented Gram-positive bacterial member of the aquatic environment and also found in fish intestinal microbiota (Jayanth *et al.*, 2001; Chabrillon *et al.*, 2005; Abd El-Rahman *et al.*, 2009). There are published data on the association of this organism with fish diseases (Austin & Stobie, 1992) and non-pathogenic strains of this species were used as probiotic against *Aeromonas salmonicida* in rainbow trout (Irianto & Austin, 2002), *Aeromonas hydrophila* in tilapia (Abd El-Rahman *et al.*, 2009; Osman *et al.*, 2010), *V. harveyi* in Senegal sole (*Solea senegalensis*) (Chabrillon *et al.*, 2005) and *L. anguillarum* in gilt-head sea bream (*Sparus aurata*) (Chabrillon *et al.*, 2006). Similarly, *M. luteus* isolates used in this study were recovered from the gut microbiota of healthy common dentex and they were determined as non-pathogenic to the rainbow trout in the pathogenicity assays. Also they showed an *in vitro* antagonistic effect against *L. anguillarum*.

Members of the genus *Bacillus* are usually found in the intestinal microbiota of fresh water and marine fish (Gatesoupe, 1999; Ghosh *et al.*, 2002). Furthermore, many non-pigmented *Bacillus* strains (for example *B. subtilis*) were selected in the probiotic research due to their antibiotic effectiveness against fish pathogens (Vaseeharan & Ramasamy, 2003). Some *Bacillus* species contain carotenoid pigments and, among them, *B. marisflavi* was identified in marine waters (Yoon *et al.*, 2003; Khaneja *et al.*, 2010). Despite it was detected in marine water samples, This study is the first record for the presence of *B. marisflavi* in the intestinal microflora of a marine fish species. *B. marisflavi* isolates which are determined to be non-pathogenic for rainbow trout also showed an *in vitro* antagonistic effect on *L. anguillarum*, which indicates the production of antimicrobials by *B. marisflavi*. It is likely the same result of a pathogen-inhibiting mechanism as that was previously reported for other *Bacillus* species (Bernan *et al.*, 1997; Ganguly & Mukhopadhyay, 2010; Austin & Austin, 2012).

As a result, in this study, yellow pigmented Gram-positive bacteria that are commonly and abundantly found in the gut microbiota of the healthy common dentex and larviculture system were identified by using biochemical and molecular methods. Furthermore, they were determined to be non-pathogenic for rainbow trout and their antagonistic affect against *L. anguillarum* was revealed. Thus, being non-pathogenic and having an inhibitory mechanism against *L. anguillarum*, *B. marisflavi* and *M. luteus* can be regarded as probiotic candidate species and maybe used in the further studies on the fish health and welfare.

## Acknowledgements

Different steps of this study were supported by Istanbul University Research Projects Fund with the project numbers 2010/2637, 23672 and 30698. The authors would like to thank to the Akuvatur Fish Farming Company for their help during the sampling studies.

## References

- Abd El-Rahman, A.M., Khattab, Y.A.E., Shalaby, A.M.E. 2009. *Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. *Fish Shellfish Immunology*, 27, 175-180.
- Abellan, E., 1999. Culture of common dentex (*Dentex dentex* L.) Present knowledge, problems and perspectives, in recent advances in Mediterranean aquaculture finfish species diversification. p. 157-168. In: *Proceedings of the Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM) Zaragoza (Spain), 24-28 May 1999*.
- Akayli, T., Erkan, M., Yardimci, R.E., Çanak, Ö., Ürkü, Ç., 2015. Interaction of gut flora and bacterial pathogens of cultured common dentex (*Dentex dentex*). *Bamidgeh*, 67, 1-7.
- Alsina, M., Blanch, A.R., 1994. A set of keys for biochemical identification of environmental *Vibrio* species. *Journal of Applied Bacteriology*, 76, 79-85.
- Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z. et al., 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acid Research*, 17 (25), 3389-3402.
- Austin, B., Stobie, M., 1992. Recovery of *Micrococcus luteus* and presumptive *Planococcus* sp. from moribund fish during an outbreak of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fry syndrome in England. *Journal of Fish Disease*, 15 (2), 203-206.
- Austin, B., Austin, D., 2012. *Bacterial Fish Pathogens Disease of Farmed and Wild Fish*, Springer, New York, 652 pp.
- Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Rapp, B.A. et al., 2000. GenBank. *Nucleic Acids Research*, 28, 1-10.
- Bernan, V.S., Greenstein, M., Maisese, W.M., 1997. Marine microorganisms as a source of new natural products. *Advances in Applied Microbiology*, 43, 57-90.
- Bhunia, A.K., Johnson, M.C., Ray, B., 1988. Purification, characterization and antimicrobial spectrum of bacteriocin produced by *Pediococcus acidilactici*. *Journal of Applied Bacteriology*, 65, 261-268.
- Bintang, M., Kusumavati, D.E., Safira, U.M., Pasaribu, F.H., Sidhartha, T., 2014. Analysis 16S rRNA sequence of endophytic bacteria isolate DM6 form *Coleus scutellariodes* (L.) Beneth. leaves. In: *International Conference on Agricultural, Environmental and Biological Sciences (AEBS-2014), Phuket (Thailand), 24-25 April 2014*.
- Chabrillon, M., Arijo, S., Diaz-Rosales, P., Balebona, M.C., Morinigo, M.A., 2006. Interference of *Listonella anguillarum* with potential probiotic microorganisms isolated from farmed gilt-head seabream (*Sparus aurata*, L.). *Aquaculture Research*, 37, 78-86.
- Chabrillon, M., Rico, R.M., Balebona, M.C., Morinigo, M.A., 2005. Adhesion to sole, *Solea senegalensis* Kaup, mucus of microorganisms isolated from farmed fish and their interaction with *Photobacterium damsela* subsp. *piscicida*. *Journal of Fish Disease*, 28, 229-237.
- Eder, W., Wolfgang, L., Huber, R., 1999. Novel 16S rRNA gene sequences retrieved from highly saline brine sediments of Kebrut Deep, Red Sea. *Archives of Microbiology*, 172, 213-218.
- Efthimiou, S., Divanach, P., Rosenthal, H., 1994. Growth, food conversion and agonistic behaviour in common dentex, *Dentex dentex*, juveniles fed on pelleted moist and dry diets. *Aquatic Living Resources*, 7, 267-275.
- Firat, K., Saka, Ş., Çoban, D., 2003. The effect of light intensity on early life development of common dentex *Dentex dentex* (L. 1758) larvae. *Aquaculture Research*, 34, 727-732.
- Ganguly, P.I., Mukhopadhyay, K.S., 2010. Application and effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture: A Review. *Bamidgeh*, 62 (3), 130-138.
- Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. *Aquaculture*, 180, 147-165.
- Ghosh, K., Sen, S.K., Ray, A.K. 2002. Characterization of bacilli isolated from the Gut of rohu, *Labeo rohita*, fingerlings and its significance in digestion. *Journal of Applied Aquaculture*, 12 (3), 33-42.
- Gomez-Gil, B., Roque, A., Turnbull, J.F., 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture*, 191, 259-270.
- Grisez, L., Reyniers, J., Verdonck, L., Swings, J., Ollevier, F., 1997. Dominant intestinal microflora of sea bream and sea bass larvae, from two hatcheries, during larval development. *Aquaculture*, 155, 387-399.
- Irianto, A., Austin, B., 2002. Probiotic in aquaculture. *Journal of Fish Disease*, 25, 633-642.
- Janda, J.M., Abbott, S.L., 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 45(9), 2761-2764.
- Jayanth, K., Jeyasekaran, G., Shakila, R.J., 2001. Biocontrol of fish bacterial pathogens by the antagonistic bacteria isolated from the coastal waters of Gulf of Mannar, India. *European Association of Fish Pathologists*, 21 (1), 12-18.
- Khaneja, R., Perez-Fons, L., Fakhry, S., Baccigalupi, L., Steiger, S. et al., 2010. Carotenoids found in *Bacillus*. *Journal of Applied Microbiology*, 108 (6), 1889-1902.
- Koumoundouros, G., Carrillo, J., Divanach, P., Kentouri, M., 2004. The rearing of common dentex *Dentex dentex* (L.) during the hatchery and on-growing phases. *Aquaculture*, 240, 165-173.
- Lane, D.J., 1991. 16S/23S rRNA sequencing. p. 115-175. In: *Nucleic Acid Techniques in Bacterial Systematics*. Stackebrandt, E., Goodfellow, M. (Eds). Wiley, Chichester.
- Lemos, M.L., Toranzo, A.E., Barja, J.L., 1985. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. *Microbial Ecology*, 11, 149-163.
- Liu, G.Y., Essex, A., Buchanan, J.T., Datta, V., Hoffman, H.M. et al., 2005. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *Journal of Experimental Medicine*, 202, 209-215.
- Muroga, K., Higashi, M., Keitoku, H., 1987. The isolation of intestinal microflora of farmed red sea bream (*Pagrus*



- major*) and black sea bream (*Acanthopagrus schlegeli*) at larval stage and juvenile stages. *Aquaculture*, 65, 79-88.
- Nair, S., Simidu, U., 1987. Distribution and significance of heterotrophic marine bacteria with antibacterial activity. *Applied Environmental Microbiology*, 53, 2957-2962.
- Osman, H.A.M., Ibrahim, T., Soliman, W., Aboud, O., 2010. Improvement growth and immune status using a potential probiotic bacteria *Micrococcus* species among cultured *Oreochromis niloticus*. *New York Science Journal*, 3 (10), 5-11.
- Rueda, F.M., Martínez, F.J., 2001. A review on the biology and potential aquaculture of *Dentex dentex*. *Reviews in Fish Biology and Fisheries*, 11, 57-70.
- Savas, S., Kubilay, A. Basmaz, N., 2005. Effect of bacterial load in feeds on intestinal microflora of seabream (*Sparus aurata*) larvae and juveniles. *Bamidgeh*, 57 (1), 3-9.
- Spanggaard, B., Huber, I., Nielsen, J., Sick, E.B., Pipper, C.B. et al., 2001. The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. *Environmental Microbiology*, 3 (12), 755-765.
- Stackebrandt, E., Goebel, B.M., 1994. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition bacteriology. *International Journal of Systematic Bacteriology*, 44 (4), 846-849.
- Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512-526.
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., Kumar, S., 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725-2729.
- Trust, T.J., Bull, L.M., Currie, B.R., Buckley, J.T., 1979. Obligate anaerobic bacteria in the gastrointestinal microflora of the grass carp (*Ctenopharyngodon idella*), goldfish (*Carassius auratus*), and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, 36 (10), 1174-1179.
- Udey, L.R., 1978. Anaerobic bacteria as possible disease agents in fish. *Marine Fisheries Review*, 40 (10), 10-12.
- Vaseeharan, B., Ramasamy, P., 2003. Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *Letter of Applied Microbiology*, 36, 83-87.
- Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W., 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, (4), 655-671.
- Wieser, M., Denner, E.B.M., Kumpfer, P., Schumann, P., Tindall, B. et al., 2002. Emended descriptions of the genus *Micrococcus*, *Micrococcus luteus* (Cohn 1872) and *Micrococcus lylae* (Kloos et al. 1974). *International Journal of Systematic and Evolutionary Microbiology*, 52, 629-637.
- Woo, P.C.Y., Teng, J.L.L., Wu, J.K.L., Leung, F.P.S., Tse, H. et al., 2009. Guidelines for interpretation of 16S rRNA gene sequence-based results for identification of medically important aerobic Gram-positive bacteria. *Journal of Medical Microbiology*, 58, 1030-1036.
- Yoon, J.H., Kim, I.G., Kang, K.H., Oh, T.K., Park, Y.H., 2003. *Bacillus marisflavi* sp. nov. and *Bacillus aquimaris* sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea. *International Journal of Systematic and Evolutionary Microbiology*, 53, 1297-1303.