Ankara Üniv Vet Fak Derg, **59**, 141-146, 2012

Investigation of the presence and antibiotic susceptibilities of *Flavobacterium psychrophilum* in rainbow trout farms (*Oncorhynchus mykiss* Walbaum, 1792) in The Middle and Eastern Black Sea Regions of Turkey*

Yüksel DURMAZ[1], Ertan Emek ONUK[2], Alper ÇİFTCİ[3]

[1]Veterinary Control and Research Institute, [2]Department of Fish Diseases and [3]Microbiology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun.

Summary: The aims of the study were to find out the presence of *Flavobacterium psychrophilum* (*F. psychrophilum*) in commercial Rainbow trout farms in Middle and Eastern Black Sea regions and to determine the antibiotic susceptibility profiles of the isolated strains. For this purpose, total of 276 fish samples were collected from 18 different farms in these regions between January 2008 and July 2010. Bacterial isolation from samples was performed by conventional microbiological methods. In addition, Polymerase Chain Reaction (PCR) was used to confirm the identification and to detect *F. psychrophilum* in tissue samples. Nine of fish tissue samples were found to be positive for *F. psychrophilum* by PCR. However, five *F. psychrophilum* strains were isolated from these samples and confirmed by PCR. The antibiotic resistance of the isolates against neomycine, oxytetracycline, enrofloxacin, amoxycillin, ampicillin, erythromycin, kanamycin, sulphamethoxazole+trimethoprim, cefoperazone and oxolinic acid were analyzed by Kirby Bauer agar disc diffusion test. The antibiotic susceptibility patterns of the isolates were variable. All the isolates were sensitive only to oxytetracyline (30 µg) and enrofloxacine (5 µg).

Key words: Antibiotic susceptibility, *F. pscychrophilum,* identification, PCR.

Orta ve Doğu Karadeniz Bölgesi gökkuşağı alabalığı çiftliklerinde (*Oncorhynchus Mykiss* Walbaum, 1792) *Flavobacterium psychrophilum* varlığının ve antibiyotik duyarlılıklarınının incelenmesi

Özet: Bu çalışma, Orta ve Doğu Karadeniz bölgesinde bulunan ticari gökkuşağı alabalığı çiftliklerinde *F. psychrophilum*'un varlığının ve antibiyotik duyarlılıklarının belirlenmesi amacıyla yapılmıştır. Çalışma kapsamında Ocak 2008-Temmuz 2010 tarihleri arasında bölgedeki 18 farklı çiftlikten toplam 276 balık örneği toplandı. Örneklerden bakteri izolasyonu konvansiyonel mikrobiyolojik metotlar ile yapıldı. Elde edilen izolatların moleküler olarak doğrulanmasında ve doku örneklerinden etkenin saptanmasında Polimeraz Zincir Reaksiyonu (PZR) kullanıldı. Balık dokularının dokuzunun PZR ile pozitif sonuç verdiği saptandı. Bu dokuların beşinden *F. psychrophilum* izole edildi ve PZR ile doğrulandı. İzolatların neomisin, oksitetrasiklin, enrofloksasin, amoksilin, ampisilin, eritromisin, kanamisin, sulfametoksazol+trimetoprim, sefoperazon ve oksolinik asit antibiyotiklerine karşı direnç durumu, Kirby Bauer agar disk diffüzyon yöntemi ile araştırıldı. Antibiyotik duyarlılık profilinin değişken olduğu görüldü. İzolatların tamamının oksitetrasiklin ve enrofloksasin antibiyotiklerine karşı duyarlı olduğu belirlendi.

Anahtar sözcükler: Antibiyotik duyarlılığı, *F. pscychrophilum,* identifikasyon, PCR.

Introduction

Flavobacterium psychrophilum is the causative agent of bacterial cold water disease (BCWD) and rainbow trout fry syndrome (RTFS) (26) which both are important infectious diseases in farmed fish (13, 21). *F. psychrophilum* is a Gram negative flexible, slender rod shaped bacteria (26) that produces non-diffusible yellow pigment on agar medium (12). *F. psychrophilum* strains generally shows a slow gliding motility (26). The optimum growth temperature of the agent is 15˚C (13) and it can not grow above 25˚C (29). The pathogen was first isolated from juvenile coho salmon (*Oncorhynchus kisutc*) in 1948 in USA (5). The causative agent of the disease has been isolated from most areas of the world such as Australia, Belgium, Canada, Chile, Denmark, Finland, France, Italy, Japan, Korea, Spain, Sweden and United Kingdom (8, 12, 26) and its economical importance in aquaculture of freshwater salmonid is

* This study was supported by Republic of Turkey, Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research (Project number: TAGEM/HS/09/02/08/140) and presented in IXth Congress of the National Veterinary Microbiology, 05-07 October 2010, Girne/Cyprus.

emphasized all over the world (24, 26). In Turkey, *F. psychrophilum* was isolated from rainbow trout farms in Aegean, Marmara, Mediterranean, Center Anatolia and Eastern Anatolia (11, 14, 18, 19). The first *F. psychrophilum* isolation from fish with rainbow trout fry has been performed by Balta (3) in Aegean region.

There is no commercial vaccine for preventing *F. psychrophilum* diseases (30). Therefore, good preventive management of disease usually depends on early and accurate diagnosis of infection and to combat *F. psychrophilum* infections, the most effective way is correct antibiotic therapy (26). There are some difficulties for detection and identification of causative agent because of its fastidious feature, lack of selective media, being weakly reactive in some biochemical tests (2, 22). Therefore the diagnosis of the disease by conventional methods based on phenotypic characteristics is laborious and time consuming. Furthermore, these techniques generally lack sensitivity to allow the detection of low levels of *F. psychrophilum* from various sources (33). While taking into consideration these disadvantages, it is clear that faster and more sensitive techniques to detect *F. psychrophilum* present in small numbers in tissues and to assess the epidemiology of agent are needed. Polymerase Chain Reaction (PCR) has been reported as a sensitive technique which is suitable to detect *F. psychrophilum* from environment and fish tissues (15, 16, 32, 33).

In Turkey, trout (inland water) is the most widely grown as coldwater fish and has the highest production rate with 47.6% in aquaculture (31). The objectives of this study were to find out the presence of *F. psychrophilum* infections in commercial rainbow trout farms in middle and eastern Black Sea regions of Turkey and to determine the antibiotic resistance profiles of isolated strains.

Materials and Methods

Sample collection: Fish samples were collected from 18 different commercial rainbow trout farms (4 farms have hatchery unit) in Samsun, Sinop, Ordu, Trabzon and Rize provinces in Middle and Eastern Black Sea regions between January 2008-July 2010. Samples were collected from ponds that were suspected to include diseased fishes. A total of 276 rainbow trout of various sizes (2 to 400 g) were taken for microbiological analyses.

Isolation and identification of F. psychrophilum by conventional methods: Cytophaga Agar (CA, 0.05% tryptone, 0.05% yeast extract, 0.02% sodium acetate, 0.02% beef extract with 0.9% agar, pH 7.2-7.4) (1) was used for culturing of *F. psychrophilum*. Samples from internal organs (spleen, liver, kidney and heart), damaged gill tissue and, if present, skin lesions of fish body surface were streaked onto CA plates directly and incubated at 18°C for 4-7 days. After incubation period, yellow-pigmented colonies were chosen and restreaked on the CA to obtain pure isolates. Colonies were tested for Gram staining, presence of flexirubin type pigment, cytochrome oxidase activity, catalase production and motility (21). Gram negative rod shaped, gliding motility, production of flexirubin type pigment were taken for identification and further characterization.

*Identification of F. psychrophilum by PCR***:** DNA was extracted from the isolates and fish tissues using a commercial extraction kit (Qiagen, Cat. No.69506) according to manufacturer's instructions. The extracted DNA was amplified using oligonucleotide primer set specific for *F. psychrophilum* (32). The sequences of two primers were FP1 (5'-GTTAGTTGGCATCAACAC-3' and FP2 (5'-TCGATCCTACTTGCGTAG-3'). For amplification, 25 µl of PCR master mix containing DEPC-treated water, 1 x PCR Buffer, 1.6 mM of $MgCl_2$, 0.2 mM of each dNTP, 1.0 U of Taq polymerase, 1µM of each primer and 5 µl of template DNA was prepared. The oligonucleotides and enzyme used for PCR assay were supplied by Fermentas Inc. (Lithuania). The amplification was carried out with the following conditions: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of amplification (denaturation at 94°C for 30 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min) and a final elongation period at 72°C for 10 min. The amplicons were transferred to 1.5% agarose gel and electrophoresed. DNA bands were stained with ethidium bromide (2 µg/ml) and visualized by an UV transilluminator. The bands at the molecular size of approximately 1088 bp were considered as positive for *F. psychrophilum*. *F. psychrophilum* ATCC (R) 93-11 49510 FXBT and *F. columnare* ATCC 49512 strains were used as positive and negative controls, respectively.

Antibiotic susceptibility test: Antibiotic susceptibility test was performed to determine the antibiotic resistant profiles of *F. psychrophilum* isolates using the Kirby-Bauer disc diffusion method (4). Antibiotic discs (Oxoid, England) of neomycin (30 µg), oxytetracycline (30 µg), enrofloxacin (5 µg), amoxicillin (10 µg), ampicillin (10 µg), erythromycin (15 µg), sulphamethoxazole+ trimethoprim (23.75+1.25 µg), kanamycin (30 µg), cefoperazone (75 µg), oxolinic acid (2 µg) were used for determining the resistance profiles. Briefly, Cytophaga Broth (CB) was used to prepare bacterial suspensions. The turbidity of suspensions was adjusted as Mac Farland 0.5 and 100 µl of aliquots were spread over CA surface. Antibiotic disks were placed on the surface of the inoculated agar plates and the plates were incubated at 18°C for 3-5 days. After incubation period, the antibiotic inhibition zone diameters were measured and the results were evaluated according to the NCCLS (25).

Results

Isolation and identification of F. psychrophilum: A total of 38 Gram negative, long and thin bacilli showing gliding movement and flexirubin type pigment production were isolated from samples (one isolate from liver and others from gills). These isolates were then tested by some biochemical tests such as catalase, cytochrome oxidase, ONPG, H_2S and esculin tests. A total of 12 (31.57%) isolates which were found as weakly positive for catalase and cytochrome oxidase, negative for ONPG, H_2S and esculin were identified as suspicious for *F. psychrophilum* and identification was confirmed by PCR.

Confirmation of the identification by PCR: In the PCR analysis of 12 isolates identified phenotypically, five were determined as positive. Moreover, in direct PCR analysis of tissue mixtures (including also the tissues from which *F. psychrophilum* strains were isolated), nine were found positive for *F. psychrophilum* (Fig.1).

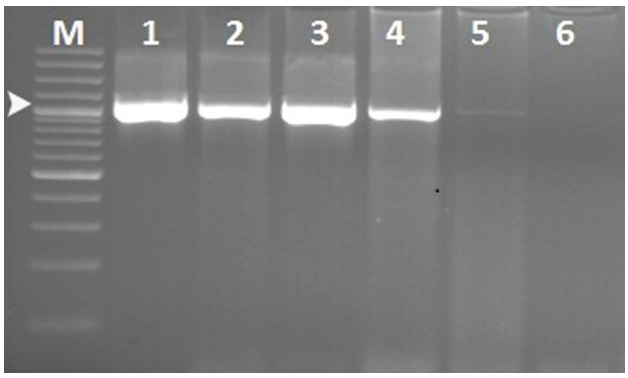

Figure 1. *F. psychrophilum* specific PCR, 1088 bp. M; Molecular weight standard (100-1500 bp) 1; *F. psychrophilum* ATCC 49510, 2-4; *F. psychrophilum* isolates, 5; *F. psychrophilum* positive tissue sample, 6; *F. columnare* ATCC 49512

Şekil 1. *F. psychrophilum* spesifik PCR, 1088 bp. M; Molecular ağırlık standardı (100-1500 bp) 1; *F. psychrophilum* ATCC 49510, 2-4; *F. psychrophilum* izolatları, 5; *F. psychrophilum* pozitif doku örneği, 6; *F. columnare* ATCC 49512

Of five isolates confirmed as *F. psychrophilum* by PCR, one was isolated from liver and four were isolated from gills. The only isolate from liver was obtained from farm 15. However, two tissue mix samples (including the sample tissue from which the isolation was performed) which were collected from farm 15 were found to be positive in the PCR analysis. All the isolates obtained from gill samples were originated from farm 17. Among 11 tissue samples in this farm, five were found to be positive for *F. psychrophilum* by PCR. Moreover, while no isolation was made from the samples in farm 13, two fish tissue samples were detected to be positive for *F. psychrophilum* by PCR (Table 1).

Table 1. Isolation and PCR detection of *F. psychrophilum* from fish samples collected from various ponds with different water temperature

Tablo 1. Farklı su sıcaklığına sahip havuzlardan toplanan balık örneklerinden *F. psychrophilum*'un izolasyonu ve PCR ile belirlenmesi

Fish farm no.	Water temperature	Sample number	Number of strains isolated from samples	Number of PCR positive tissue samples
1	19.5	7	0	0
2	11.7	15	0	0
3	11.8	27	0	0
4	13.0	16	0	0
5	9.2	25	0	0
6	7.5	24	0	0
7	10.5	18	0	0
8	9.6	14	0	0
9	12.1	12	0	0
10	9.3	13	0	0
11	9.2	19	0	0
12	11.7	22	0	0
13	6.2	15	0	2
14	8.0	16	0	0
15	7.2	6	1	2
16	15.0	8	0	0
17	8	11	4	5
18	13.3	8	0	0
Total		276	5	9

Table 2. Antibiotic susceptibility profiles of *F. psychrophilum* isolates

Tablo 2. *F. psychrophilum* izolatlarının antibiyotik duyarlılık profilleri

	Strain No				
Antibiotic Disc	1	2	3	4	5
Neomycin 30 µg	R	R	R	R	R
Oxytetracyclin 30 µg	S	S	S	S	S
Enrofloxacin 5 µg	S	S	S	S	S
Amoxicillin 10 µg	R	R	R	R	R
Ampicillin 10 µg	R	R	R	R	R
Erythromycin 15 µg	S	R	R	R	R
Kanamycin 30 µg	R	R	R	R	R
Cefoperazone 75 µg	R	S	S	S	R
Oxolinic acid 2 µg	S	S	R	S	S
Sulphamethoxazole+ Trimetoprim 23.75+1.25 µg	S	S	R	S	S

R, resistant; S, susceptible

The primer pair of FP1 and FP2 generated a fragment of identical size (1088 bp) from all tested strains of *F. psychrophilum* including the reference strain *F. psychrophilum* ATCC (R) 93-11 49510 FXBT. This specific band was not detected when DNA from *F. columnare* ATCC 49512 strain was used.

Antibiotic susceptibility test: All five (100%) *F. psychrophilum* isolates were found to be sensitive to oxytetracyclin and enrofloxacin, but resistant to kanamycin, ampicillin, amoxicillin and neomycin.

Among these isolates only one strain (strain no 1) (20%) was sensitive to erythromycin. On the other hand, only one isolate (strain no 3) (20%) was found to be resistant to oxolinic acid and sulphamethoxazole+trimetophrim. Besides, two isolates (strain no 1 and 5) (40%) were resistant to cefoperazone (Table 2).

Discussion and Conclusion

RTFS and BCWD caused by *F. psychrophilum* are responsible for significant economic losses in salmonid culture (26). In Turkey, *F. psychrophilum* has first been isolated from rainbow trout in 1997 (3). Later, the agent has shown wide distribution among various geographical regions of Turkey (6, 10, 11, 17, 18, 19, 20).

In Turkey, there is no specific regional study investigating the epidemiology of *F. psychrophilum*. However, in a local survey that has been performed in South of the Black Sea, only one (0.2%) *F. psychrophilum* has been isolated from 558 fish samples by conventional microbiological methods (17). In the present study, five (1.8%) *F. psychrophilum* strains were isolated from 276 fish samples in farms located in the Middle and Eastern Black Sea. Furthermore, *F. psychrophilum* was identified in nine (3.2%) tissue samples by PCR. These results indicate that *F. psychrophilum* was presented at low levels in these regions. However, when compared to the year 2009, it was observed that the prevalence of the agent in those regions is tends to increase. Therefore, large-scaled studies understanding epidemiology of the agent and developing effective control strategies are needed.

Several studies (12, 23) have shown that *F. psychrophilum* strains are highly homogeneous phenotypically. In this study, also it was observed that all five isolates showed high level of homogeneity in their biochemical and morphological characteristics. Moreover, the phenotypic characteristics of these strains were similar to those identified in previous studies (21, 23, 34).

The growing economic importance of aquaculture in the world has led to increase interest in the rapid and reliable methods for detection and identification of bacterial fish pathogens (27). The detection of *F. psychrophilum* by conventional techniques is difficult and time-consuming. These methods have also relatively low sensitivity for detection of the agent especially in carrier fish. PCR, one of the molecular based methods, is used as a specific, sensitive and rapid method to detect the agent in fish tissues. Wiklund et al. (33) have reported that a PCR protocol using PSY1 and PSY2 primers for detection of *F. psychrophilum* in infected rainbow trout was more sensitive than agar culture. Urdaci et al. (32), have designed two primers, FP1 and FP2, considering two variable regions in 16S rRNA sequence for detection of *F. psychrophilum*. The specifity of these primers has been determined using different *F. psychrophilum* strains and taxonomically related species. They have observed a 1088 bp product specific for *F. psychrophilum*. Similarly, in the PCR analysis based on FP1 and FP2 primers which were used to confirm the identification of *F. psychrophilum* strains and also to detect the agent in fish tissues directly, amplification products at the size of approximately 1088 bp were observed in current study. Five isolates were confirmed as *F. psychrophilum* and the agent was detected in four fish tissues also in addition to those from which the isolations were made. Michel et al. (24) have reported that in some cases, isolation was not possible from infected tissues due to the presence of viable but non-cultivable cells. In this study, this may be one of the reasons why we could not isolate *F. psychrophilum*, though the agent was detected in tissues by PCR in farm 13. Furthermore, it should not be ignored that unconscious use of antibiotics in fish farms may lead to inhibit the bacterial growth. The results in the present study also showed that PCR was more sensitive than conventional microbiological methods and it can be used to detect *F. psychrophilum* which is difficult to culture and to detect carrier fishes especially.

Several studies have been performed to determine the antibiotic resistance profiles of *F. psychrophilum* in various regions of Turkey and quite variable profiles have been observed. Balta (3) has reported that *F. psychrophilum* was sensitive to nitrofurans but resistant to flumequin, sulphanamids and oxolinic acid. Diler et al. (11) have reported that, two *F. psychrophilum* isolates were sensitive to amoxicillin-clavulanic acid, oxytetracycline and gentamicin but resistant to trimethoprim. In another study, five *F. psychrophilum* strains isolated from rainbow trout farms (five different farms) in Eastern Anatolia have been reported to be sensitive to oxytetracycline, erythromycin, gentamicin, nitrofuran and amoxicillin-clavulanic acid, but resistant to chloramphenicol and penicillin (14). Boyacıoğlu (6) has reported that 20 *F. psychrophilum* strains isolated from an outbreak in Muğla province are resistant to ampicillin (95%), sulphamethoxazole (95%), erythromycin (45%) and oxytetracycline (20%) but sensitive to enrofloxacin (100%). Different techniques have been used to evaluate antibiotic resistance profile in several studies. In Mediterranean Region, 13 *F. psychrophilum* strains isolated from different outbreaks have been found to be resistant to enrofloxacin (23%), amoxicillin (46.2%), erythromycin (61.5%), kanamycin (30.8%), cefoperazone (46.2%) and oxolinic acid (53.8%) using MINI API system (10). Kum et al. (20) have determined the antibiotic resistance profiles of 20 *F. psychrophilum* strains isolated from four different hatcheries in Eastern Aegean both by disc diffusion and agar dilution techniques and have found the discrepancies between testing methods. In this study, all *F. psychrophilum*

isolates were found to be resistant to neomycin, ampicillin, amoxicillin and kanamycin. Furthermore, 80%, 40%, 20% and 20% of these isolates were found to be resistant to erythromycin, cefaperazone, oxolinic acid and sulphamethaxazole+trimetoprim, respectively. However all the strains were 100% sensitive to oxytetracycline and enrofloxacin. It was considered that these variable resistance profiles may be due to the widely use of different antibiotics or to differences among the strains.

Several studies carried out in different countries have also showed the differences in resistance profiles among *F. psychrophilum* isolates. Valdebenito and Avendano-Herrera (34) have found that 20 *F. psychrophilum* isolates from Chile were resistant to sulphamethoxazole+trimethoprim, but highly sensitive to amoxicillin using agar disc diffusion test. In Denmark, 387 *F. psychrophilum* isolates have been found to be resistant to amoxicillin (11.6%), oxolinic acid (65.9%), oxytetracycline (67.7%) and sulphamethoxazole+ trimethoprim (98.2%) in microdilution test (7). In another study (9), 25 *F. psychrophilum* isolates originated from Spain have been found as resistant to oxytetracycline (>80%) by using broth macrodilution method. The findings of the present study were not in agreement with these of the above mentioned. However, Rangdale et al. (28) have reported the similar results to the current study with respect to sensitivity to enrofloxacin. The differences in antibiotic resistance profiles of *F. psychrophilum* may be due to the use of different techniques or strain variations.

In conclusion, *F. psychrophilum* was detected in commercial rainbow trout farms in the Middle and the Eastern Black Sea by both culture and PCR techniques. The antibiotic resistance profiles of the isolates were determined via disc diffusion tests. It was concluded that the PCR was more robust than cultural techniques especially for screening and detection of *F. psychrophilum*. Furthermore, to prevent possible economic losses due to *F. psychrophilum* infections, a monitoring program should be undertaken.

References

1. **Anacker RL, Ordal EJ** (1959): *Study on the myxobacterium Chondrococcus columnaris. I. Serological typing*. J Bacteriol, **78,** 25-32.
2. **Baliarda A, Faure D, Urdaci MC** (2002): *Development and application of a nested PCR to monitor brood stock salmonid ovarian fluid and spleen for detection of the fish pathogen Flavobacterium psychrophilum*. J Appl Microbiol, **92,** 510-516.
3. **Balta F** (1997): *Kültürü yapılan alabalıklarda (Oncorhynchus mykiss) görülen Flexibacter psychrophila enfeksiyonu*. IX. Ulusal Su Ürünleri Sempozyumu. 17-19 Eylül 1997. Eğirdir/Isparta. 641-648.
4. **Bauer AW, Kirby WM, Sherris JC, Turck M** (1966): *Antibiotic susceptibility testing by a standardized single disk method*. Am J Clin Pathol, **45 (4),** 493-496.
5. **Borg AF** (1960): *Studies on myxobacteria associated with diseases in salmonid fishes*. American Association for the Advancement of Science, Wildlife Disease. Washington, DC. **8,** 1-85.
6. **Boyacıoğlu M** (2007): *Gökkuşağı alabalıklarında (Oncorhynchus Mykıss) RTFS'ye (Rainbow Trout Fry Syndrome) neden olan Flavobacterıum Psychrophilum etkeninin izolasyonu ve antibakteriyel sağaltım seçeneğinin belirlenmesi*. T.C. Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmakoloji ve Toksikoloji Anabilim Dalı, AYDIN.
7. **Bruun MS, Schmidt AS, Madsen L, Dalsgaard I** (2000): *Antimicrobial resistance patterns in Danish isolates of Flalobacterium psychrophilum.* Aquaculture, **187,** 201-212.
8. **Cipriano RC, Holt RA** (2005): *Flavobacterium psychrophilum, cause of Bacterial Cold-Water Disease and Rainbow Trout Fry Syndrome*. Fish Disease Leaflet No. 86. United States Dept. of the Interior. U.S. Geological Service, National Fish Health Research Laboratory, Kearneysville, WV.
9. **Del Cerro A, Marquez I, Prieto JM** (2010): *Genetic diversity and antimicrobial resistance of Flavobacterium psychrophilum isolated from cultured rainbow trout, Onchorynchus mykiss (Walbaum), in Spain*. J Fish Dis, **33,** 285-291.
10. **Didinen BI, Diler O, Ekici S, Altun S** (2005): *Flavobacterium psychrophilum izolatlarının teşhisinde API ZYM kullanımı ve ATB VET ile antimikrobiyal duyarlılığın belirlenmesi*. SDÜ Eğirdir Su Ürünleri Fakültesi Dergisi, **1,** 64-70.
11. **Diler Ö, Altun S, Işıklı BI** (2003): *Kültürü yapılan gökkuşağı alabalığı (Oncorhynchus mykiss)'ndan izole edilen Flavobacterium psychrophilum'un fenotipik karakterleri*. SDÜ Fen Bilimleri Enstitüsü Dergisi, **7,** 1-8.
12. **Hesami S, Allen KJ, Metcalf D, Ostland VE, MacInnes JI, Lumsden JS** (2008): *Phenotypic and genotypic analysis of Flavobacterium psychrophilum isolates from Ontario salmonids with bacterial coldwater disease*. Can J Microbiol, **54 (8),** 619-629.
13. **Holt RA, Rohovec JS, Fryer JL** (1993): *Bacterial cold-water disease*. 3-23. In: V Inglis, RJ Roberts, NR Bromage (Ed), Bacterial Diseases of Fish. Blackwell Scientific Publications, Oxford.
14. **İspir Ü, Şeker E, Sağlam N, Dörücü M** (2004): *Doğu Anadolu bölgesinde bazı gökkuşağı alabalığı (Oncorhynchus mykiss) işletmelerinde görülen Flavobacterium psychrophilum enfeksiyonun araştırılması*. F Ü Fen ve Müh Bil Derg, **16 (4),** 718-724.
15. **Izumi S, Fujii H, Aranishi F** (2005): *Detection and identification of Flavobacterium psychrophilum from gill washings and benthic diatoms by PCR-based sequencing analysis*. J Fish Dis, **28,** 559-564.
16. **Izumi S, Wakabayashi H** (1997): *Use of PCR to detect Cytophaga psychrophila from apparently healthy juvenile ayu and coho salmon eggs*. Fish Pathol, **32,** 169-173.
17. **Kayis S, Capkin E, Balta F, Altinok I** (2009): *Bacteria in Rainbow Trout (Oncorhynchus mykiss) in the Southern Black Sea Region of Turkey-A Survey*. Isr J Aquacult-Bamid, **61 (4),** 339-344.
18. **Korun J, Timur G** (2001): *Gökkuşağı alabalıklarında (O. mykiss) mortalite sendromu (FMS) üzerinde bir çalışma*. İstanbul Üniversitesi. Su Ürünleri Dergisi, **12,** 15-30.

19. **Kubilay A, Altun S, Didinen BI, Ekici S, Diler Ö** (2009): *Gökkuşağı Alabalığı (Oncorhyncus mykiss) İşletmelerinde Flavobacterium psychrophilum İzalosyonu*. Kafkas Univ Vet Fak Derg, **15 (5),** 709-715.
20. **Kum C, Kırkan S, Sekkin S, Akar F, Boyacıoğlu M** (2008): *Comparison of In Vitro Antimicrobial Susceptibility in Flavobacterium psychrophilum Isolated from Rainbow Trout Fry*. J Aquat Anim Health. **20,** 245-251.
21. **Lorenzen E, Dalsgaard I, Bernardet JF** (1997): *Characterization of isolates of Flavobacterium psychrophilum associated with coldwater disease or rainbow trout fry syndrome I: Phenotypic and genomic studies*. Dis Aquat Org, **31,** 197-208.
22. **Lorenzen E, Karas N** (1992): *Detection of Flexibacter psychrophilus by immunofluorescence in fish suffering from fry mortality syndrome: a rapid diagnostic method*. Dis Aquat Org, **13,** 231-234.
23. **Madetoja J, Hanninen M-L, Hirvela-Koski V, Dalsgaard I, Wiklund T** (2001): *Phenotypic and genotypic characterization of Flavobacterium psychrophilum from Finnish fish farms*. J Fish Dis, **24,** 469-479.
24. **Michel C, Antonio D, Hedrick RP** (1999): *Production of viable cultures of Flavobacterium psychrophilum approach and control*. Res Microbiol, **150,** 351-358.
25. **National Committee for Clinical Laboratory Standards** (1999): *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals*. National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, Approved Standard M 31-A19 (11).
26. **Nematollahı A, Decostere A, Pasmans F, Haesebrouck F** (2003): *Flavobacterium psychrophilum infections in salmonid fish*. J Fish Dis, **26,** 563-574.
27. **Nilsson WB, Strom MS** (2002): *Detection and identification of bacterial pathogens of fish in kidney tissue using terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes*. Dis Aquat Organ, **48,** 175-185.
28. **Rangdale RE, Richards RH, Alderman DJ** (1997): *Minimum inhibitory concentrations of selected antimicrobial compounds against Flavobacterium psychrophilum the causal agent of rainbow trout fry syndrome (RTFS)*. Aquaculture, **158,** 193-201.
29. **Shotts EB, Starliper CE** (1999): *Flavobacterial Diseases: Columnaris Disease, Cold-water Disease and Bacterial Gill Disease*. 559-576. In: PTK Woo, DW Bruno (Ed), Fish Diseases and Disorders Volume 3. Viral, Bacterial and Fungal İnfections. CAB International, New York, USA.
30. **Stenholm AR, Dalsgaard I, Middelboe M** (2008): *Isolation and Characterization of Bacteriophages Infecting the Fish Pathogen Flavobacterium psychrophilum*. Appl Environ Microbiol, **74 (13),** 4070-4078.
31. **Turkish Statistical Institute** (2010): *Fishery Statistics 2009*. Turkish Statistical Institute Printing Division, ANKARA.
32. **Urdaci MC, Chakroun C, Faure D, Bernardet JF** (1998): *Development of a polymerase chain reaction assay for identification and detection of the fish pathogen Flavobacterium psychrophilum.* Res Microbiol, **149,** 519-530.
33. **Wiklund T, Madsen L, Bruun M.S, Dalsgaard I** (2000): *Detection of Flavobacterium psychrophilum from fish tissue and water samples by PCR amplification*. J Appl Microbiol, **88,** 299-307.
34. **Valdebenito S, Avendano-Herrera R** (2009): *Phenotypic, serological and genetic characterization of Flavobacterium psychrophilum strains isolated from salmonids in Chile*. J Fish Dis, **32,** 321-333.

Geliş tarihi: 28.04.2011 / Kabul tarihi: 18.10.2011

Address for correspondence:
Dr. Ertan Emek ONUK
Department of Fish Diseases,
Faculty of Veterinary Medicine,
University of Ondokuz Mayis,
55139 Kurupelit, Samsun–TURKEY
e-mail: eeonuk@omu.edu.tr