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A Bacterial Analysis of Some Fish Caught in the Bingöl Region of the Murat River

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Abstract: The present study investigated the bacteria present in the gills and intestines of three fish species (*Chondrostoma regium*, *Capoeta trutta* and *Cyprinion macrostomum*) obtained live from fishermen fishing in the Bingöl region of the Murat River. A total of 52 fish were sampled for analysis, with gill and intestinal tissues collected from each fish. Samples were diluted with Ringer's solution and cultured on Nutrient agar, Tryptic soy agar, Salmonella-Shigella agar, Baird-Parker agar, McConcey agar, GSP (Aeromonas and Pseudomonas selective) agar and Cytophaga agar. Bacterial identification was carried out using the application of straightforward and widely-utilised morphological analysis and biochemical tests within the field of microbiology. A total of 285 bacteria (117 from the gills and 168 from the intestines) belonging to 10 genera were isolated and identified. The most common bacterial species identified were *Aeromonas hydrophila* (12.63%), *Corynebacterium* sp. (1.05%), *Enterobacter aerogenes* (16.49%), *Escherichia coli* (35.40%), Cytophaga/Flexibacter. (4.21%), *Proteus vulgaris* (2.10%), *Salmonella* sp. (2.10%), *Streptococcus* sp. (8.77%), *Staphylococcus* sp. (14.22%) and *Vibrio* sp. (1.05%). It is very importance to carry out bacteriological examinations of fish living in all inland waters, as this provides valuable information on fish and human health, as well as offering information on water pollution through the bioindicator properties of bacteria.

Keywords: Murat River, fish, bacterial flora, gill, gut

Murat Nehrinin Bingöl Bölgesinde Yakalanan Bazı Balıkların Bakteriyel Analizleri

Özet: Bu çalışmada, Murat Nehrinin Bingöl bölgesinden avlanan balıkçılardan canlı olarak temin edilen 3 balık türünün (*Chondrostoma regium*, *Capoeta trutta* ve *Cyprinion macrostomum*) solungaç ve bağırsaklarında bulunan bakteriyeler incelendi. Elli iki balıktan solungaç ve bağırsak örneği alındı. Bu örnekler ringer çözeltisi ile dilue edilerek Nutrient agar, Triptik soy agar, Salmonella-Shigella agar, Baird-Parker agar, McConcey agar, GSP (Aeromonas ve Pseudomonas selektif) agar ve Cytophaga agar'a ekimler yapıldı. Bakteriyel identifikasyonunda basit ve yaygın olarak mikrobiyolojide kullanılan morfolojik analizler ve biyokimyasal testler ile gerçekleştirildi. Toplamda 10 cinse ait 285 bakteri (117 adet solungaç ve 168 adet bağırsak) izole ve identifiye edildi. Balıkların solungaç ve bağırsaklarında *Aeromonas hydrophila* (%12,63), *Corynebacterium* sp. (%1,05), *Enterobacter aerogenes*

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(%16,49), *Escherichia coli* (%35,40), Cytophaga/Flexibacter (%4,21), *Proteus vulgaris* (%2,10), *Salmonella* sp. (% 2,10), *Streptococcus* sp. (%8,77), *Staphylococcus* sp. (%14,22) ve *Vibrio* sp. (%1,05) türlerine rastlandı. Tüm iç sularımızda yaşayan balıklarda bakteriyolojik incelemelerin yapılması hem balık ve insan sağlığı ve hem de bakterilerin biyoindikatör özelliklerinden dolayı suyun kirliliği hakkında bilgi vermesi nedeniyle oldukça önemlidir.

Anahtar kelimeler: Murat Nehri, balık, bakteriyel flora, solungaç, bağırsak

INTRODUCTION

In addition to their use in daily activities, inland waters are employed extensively used in the agricultural, industrial, and energy sectors, and serve as habitats for a wide range of species (Anonymous, 1993). In parallel with the growth of the human population, two main strategies are been adopted to meet the increasing demand for: the opening up of more forest areas to agriculture and the use of more chemical fertilisers and pesticides on existing agricultural areas. The opening of forest areas to agriculture has been shown to cause irreversible changes in the climate and to disrupt natural fresh water cycles. In addition, increasing amounts of wastewater and chemicals used in industry and agriculture have been identified as contributing to the degradation of inland water quality (Grobler, 1989; Heath, 1995; Dörücü et al., 2023).

The severity of infection that pathogenic bacteria can cause in the aquatic environment and at higher trophic levels up to humans depends on the density of the bacteria, their adaptation to the environment, and the immune system of the infected organism (Golas et al., 2002; Doğan and Köprücü, 2023).

The development of aquaculture has resulted in bacterial fish diseases have become a major cause of economic loss (Munro, 1982; Doğan and Önalan, 2023). A significant number of pathogens are present at low concentrations in their natural environment. In general, environmental factors must also be effective for an active disease to occur in a population (Mungo and Hastings, 1993). However, the negative impact of environmental factors makes it difficult, if not impossible, to implement effective disease prevention and control measures (Şeker et al., 2006).

The bacterial flora of fish has been reported predominantly aerobic or facultatively anaerobic. These bacteria are psychrophilic, gram-negative rod-shaped bacteria of the genera Pseudomonas, Alteromonas, Moraxella, Acinetobacter, Flavobacterium, Cytophaga and Vibrio (Cahill, 1990).

It has been proposed that the bacterial flora present in fish may serve as an indicator of the bacteriological status of water, thereby providing an insight into the extent of bacterial water pollution (Geldreich and Clarke, 1966; Apun et al., 1999). It is therefore important to study the bacterial flora in fish. The aim of this study was to determine the bacterial flora of the gills and intestines of various fish species caught in the vicinity of the Genç district of Bingöl province on the Murat River, and to evaluate them in terms of their potential impact on fish and the public health.

MATERIALS and METHODS

Study Area and Fish Samples

The study was carried out on three fish species: *Chondrostoma regium*, *Capoeta trutta* and *Cyprinion macrostomum*. The fish were caught from the vicinity of the Genç district of the Bingöl province (Figure 1) of the Murat River (Table 1). The fish were obtained from local fishermen between February and May 2013. They were transported to the laboratory under anaesthesia and then euthanised with a high dose of benzocaine. The whole body was then disinfected with 70% alcohol, and the gills and intestines were sampled for cultivation by making incisions in the operculum and abdominal region under sterile conditions for bacterial isolation.

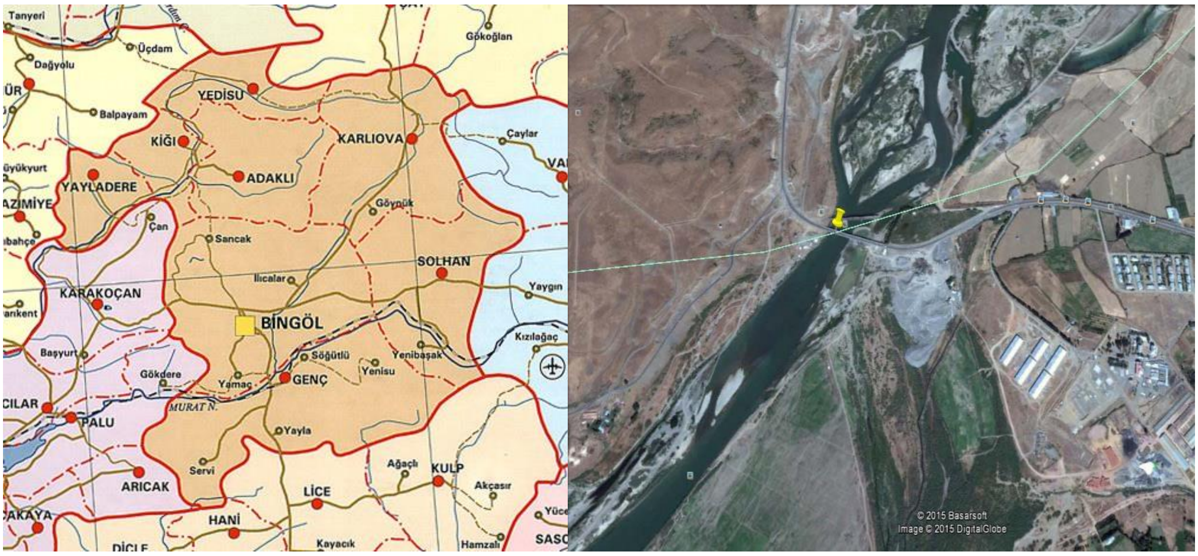

Figure 1. Study region.

Table 1. Fish species and numbers examined in the study.

Fish species	**Number**
Chondrostoma regium	11
Capoeta trutta	27
Cyprinion macrostomum	14
Total	52

Bacterial Examination

After euthanasia, samples were taken from the gills and intestines under sterile conditions and diluted with Ringer's solution. These samples were then cultured on a range of agar plates, including Nutrient, Tryptic Soy, Salmonella-Shigella, Baird-Parker, McConkey, GSP (Aeromonas and Pseudomonas selective) and Cytophaga agar plates. The Petri dishes were incubated at 25°C for a period of between 24 hours to five days. Individual colonies dropped from the main culture were then randomly selected and transferred to tryptic soy broth for enrichment. The purified strains were passaged back onto the agar on which they had been grown, and identification tests were performed using classical microbiological methods (Austin and Austin, 1993). Identification was carried out according to the methodology described in Bargey's Manual of Determinative Bacteriology (Holt et al., 1994).

RESULTS

A total of 285 bacteria were isolated and identified, comprising 117 from the gills and 168 from the intestines. Table 2 shows the number and species of bacteria obtained from both organs according to fish species. The isolated bacteria were found to belong to 10 different genera. The most common species identified in the gills and intestines were *Escherichia coli* (35.40%), *Staphylococcus* sp. (17.10%), *Enterobacter aerogenes* (16.49%) and *Aeromonas hydrophila*. (12.63%). The majority of *Escherichia coli* isolates were obtained from the gills and intestinal flora. The bacterial isolates from the intestine represented 41.05% of the total number of isolates, while the isolates from the gills represented 58.95% of the total number of isolates.

Table 2. Number and types of bacteria obtained from both organs according to fish species.

Bacteria species	Gill	Intestine	Total	Rate (%)
Aeromonas hydrophila	17	19	36	12.63
Corynebacterium sp.	3	-	3	1.05
Enterobacter aerogenes	5	42	47	16.49
Escherichia coli	40	61	101	35.40
Cytophaga/Flexibacter	4	8	12	4.21
Proteus vulgaris	2	4	6	2.10
Salmonella sp.	-	6	6	2.10
Staphylococcus sp.	30	19	49	17.10
Streptococcus sp.	16	9	25	8.77
Vibrio sp.	2	1	3	1.05
Total	117	168	285	100

DISCUSSION and CONCLUSION

In this study, bacteria were isolated and identified by culture method from 285 samples (117 gills and 168 intestines) of 52 fish (*Chondrostoma regium*, 11; *Capoeta trutta*, 27; *Cyprinion macrostomum*, 14) collected from the Bingöl region of the Murat River.

The digestive tract of freshwater fish is mainly inhabited by the bacterial species Aeromonas, Plesiomonas, and members of the Enterobacteriaceae family. It is postulated that these bacterial groups have a wide distribution in the freshwater environment and are capable of growth and multiplication under the selective conditions of the fish digestive tract. Other bacterial genera, including Acinetobacter, Bacillus, Flavobacterium, Micrococcus, Moraxella and Pseudomonas, have their origin in the water and foot. In some cases, Pseudomonas or *Acinetobacter* spp. have been identified as the predominant component of the intestinal microflora. Such conditions have been identified as indicative of suboptimal physiological conditions, including starvation and low water temperature (Austin and Austin, 1989). The following bacterial species were identified in this study: *Aeromonas hydrophila* (12.63%), *Corynebacterium* sp. (1.05%), *Enterobacter aerogenes* (16.49%), *Escherichia coli* (35.40%), Cytophaga/Flexibacter (4.21%), *Proteus vulgaris* (2.10%), *Salmonella* sp. (2.10%), *Staphylococcus* sp. (17.10%), *Streptococcus* sp. (8.77%), and *Vibrio* sp. (1.05%). The identification results of the 285 isolates in this study (Table 2) are consistent with those reported in previous studies (Austin and Austin, 1989; Holt et al., 1993; Balta, 1997; Şeker et al., 2006; Kılıç et al., 2007).

Bacteria belonging to the genus Aeromonas are naturally occurring inhabitants of aquatic environments and are recognised as a significant threat to the sustainability of aquaculture systems (Barger et al., 2021). In fish infected with *Aeromonas hydrophila*, erosive and ulcerative lesions were observed, particularly on the caudal and dorsal fins, with evidence of haemorrhaging in the underlying tissues. Furthermore, enlargement, discolouration, haemorrhages and miliary necrosis were observed in the liver. Additionally, enlargement of the spleen and kidney, hyperemia, and petechial hemorrhages in the stomach, pyloric sacs, and intestine, as well as a small amount of reddish exudate in the abdominal cavity, was observed (Sağlam et al., 2006). The same findings were observed in trout farms where this research was conducted.

A number of opportunistic pathogenic bacteria, including Corynebacterium, have been identified in freshwater fish (Arda, 1974). A review of the literature shows that Sarıeyyüpoğlu (1984) conducted a study in which the stomach and intestinal flora of 100 rainbow trout were examined for aerobic microorganisms. It was reported that 961.1% of the isolates from the gastric and intestinal flora of trout were identified as Corynebacterium. In this study, 1.05% of the isolates were identified as *Corynebacterium* sp. Corynebacterium is typically isolated during routine investigations of the microbiological quality of fish and water from freshwater fish farms (Austin and Austin, 1985; Toranzo et al., 1985; Cahill, 1990).

An isolate of *Enterobacter aerogenes* was obtained from cage-reared channel catfish with enteritis in Nanning, China (Cao et al., 2017). In the present study, the bacterium *Enterobacter aerogenes* (16.49%) was identified, and this bacterium has begun to appear in other fish species.

Escherichia coli is not a naturally occurring member of the fish microbiota; however, it can be transferred to fish through polluted water environments (Guzmán et al., 2004; Cardozo et al., 2018). In this study, the highest prevalence of *Escherichia coli* (35.40%) was observed in fish gills and intestines of fish, indicating the presence of water pollution.

The Cytophaga/Flexibacter species are microorganisms present in the natural flora of fish, but which can become pathogenic under favourable conditions (Trust, 1975). The identification of Cytophaga/Flexibacter (4.21%) in this study is consistent with the findings of Bell et al. (1971) and Yoshimizu et al. (1980).

Proteus vulgaris is a natural flora element that can be found in the digestive tract of fish, especially in the intestines (Svanevik and Lunestad, 2011). *Proteus vulgaris* has been from freshwater Nile tilapia (*Oreochromis niloticus*) from experimental freshwater aquaculture in Brazil (Boari et al., 2008) and from tilapia in Lake Victoria, Kenya (Onyango et al., 2009). In this study, *Proteus vulgaris* represented 2.10% of the 285 strains obtained, indicating that this bacterium is predominant present in the bacterial flora of freshwater fish.

Salmonella is not typically a fish pathogen; rather, the consumption of feed and water contaminated with Salmonella causes this infection (Bibi et al., 2015). The isolation of 2.10% *Salmonella* sp. in this study provides evidence of contamination with pathogen in the Bingöl region of the Murat River.

Staphylococcus sp. is not part of the natural flora of fish and other aquatic products. However, these bacteria can be isolated from freshly caught fish, especially in warm waters (Rivera et al., 2017). The high prevalence of 17.10% observed in this study can be attributed to the physicochemical characteristics of the aquatic environment and the storage and marketing conditions (Huicab-Pech et al., 2017).

Vibrio sp. is a naturally occurring bacterium that can be found in a variety of locations within the fish host, including the gastrointestinal tract, skin surface, gills, and other internal organs (Egerton et al., 2018). In this study, at least 1.05% of *Vibrio* sp. was isolated from the gills and intestines of fish, indicating low environmental stressors are strong immunity (Peterman and Posadas, 2019).

The results of this study were found to be consistent with the results of bacteriological surveys of fish from other geographical regions. It has been previously documented that the isolated bacteria are part of the normal flora of fish. Nevertheless, bacteriological examinations of fish in all inland waters are of great importance, as they provide valuable insights into fish and human health, as well as water pollution, due to the bioindicator properties of bacteria.

Ethical Approval

All animal studies were approved by the Animal Ethics Committee of KSÜZİRHADYEK and Research Institute (Protocol number: 2014/6-1).

Conflict of Interest

The authors declare that there are no conflicts of interest with other persons or organizations related to this article.

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