

Isolation of Aeromonas Strains from the Intestinal Flora of Atlantic Salmon (*Salmo salar* L. 1758)

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Abstract: The aim of this study was to isolate and identify motile Aeromonas strains from the intestinal micro-flora of healthy Atlantic salmon (*Salmo salar* L. 1758) from freshwater areas and from the Black Sea, to determine the opportunistic pathogen of motile Aeromonad septicemia in summer months. An additional aim was to compare the API 20E multiple test system with conventional biochemical methods for strains of motile Aeromonas isolated from fish. Atlantic salmon (*S. salar*) were obtained from a private-sector farm on the Black Sea coast of Turkey in summer 1995 and by the end of the examination of 51 *S. salar* intestinal flora, six motile Aeromonas strains from freshwater and two motile Aeromonas strains from the Black Sea had been isolated. Five of the motile Aeromonas strains isolated from freshwater and one of the motile Aeromonas strains isolated from the Black Sea were identified as *A. caviae*. The rest were identified as *A. sobria*.

Key Words: Aeromonas, *Salmo salar*, intestinal micro-flora

Atlantik Salmon'unun (*Salmo salar* L. 1758) Bağırsak Florasından Aeromonas Cinsi Bakterilerin İzolasyonu

Özet: Bu çalışmanın amacı, tatlısu ve Karadeniz ortamında sağlıklı salmon balıklarının (*Salmo salar* L. 1758) bağırsak florasındaki hareketli Aeromonas cinsine ait suşları izole ederek tanımlamaktır. Böylece Karadeniz koşullarında yaz aylarında deniz suyu sıcaklığının artışı ile paralel olarak ortaya çıkan hareketli Aeromonas septisemisinin olası oportunistik etkeninin varlığını saptamaktır. Ayrıca bu çalışmada balıklardan izole edilen hareketli Aeromonas türleri API 20E ve standart tüp metodları ile karşılaştırılmıştır. Atlantik salmonları, 1995 yılında Türkiye'nin Karadeniz kıyılarında bulunan ticari bir işletmeden alınmıştır. 51 adet *Salmo salar*'ın bağırsak florasının incelenmesi sonunda sekiz hareketli Aeromonas izole edilmiştir. Tatlısu örneklerinden altı, Karadeniz örneklerinden iki hareketli Aeromonas suşu izole edilmiştir. Tatlısu örneklerinden izole edilen beş ve Karadeniz örneklerinden izole edilen bir hareketli Aeromonas suşu *A. caviae* olarak tanımlanmıştır. Kalanların hepsi *A. sobria* olarak tanımlanmıştır.

Anahtar Sözcükler: Aeromonas, *Salmo salar*, bağırsak florası

Introduction

Aeromonads and Pseudomonads are characteristically bacteria from an aquatic environment. The motile Aeromonad species are often ubiquitous members of the aquatic ecosystem, but all can be components of the microbial flora of aquatic animals and may be pathogens of poikilotherms, homoiotherms and even humans (1-4). *A. hydrophila*, *A. caviae* and *A. sobria* have all been isolated from fish with the clinical signs of bacterial septicemia (2,5,6). Although it has been reported that for this disease to occur water temperature and environmental factors such as crowding and handling, as

well as low dissolved oxygen and nitrite levels, have to be adverse, the pathogenic mechanism of aeromonads is not known (5,7).

Atlantic salmon farming started on the Black Sea coast of Turkey in 1988. As salmon are not native to Turkey, the eggs were imported from Norway (8) and raised in incubators at the Sapanca Hatchery of the Fisheries Faculty of İstanbul University from where they were transported in tanks to the Black Sea and placed in cages for raising. The Black Sea appeared appropriate for salmon culture because of its low salinity and convenient temperature, but in 1991 heavy mortalities due to

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bacterial infections occurred when the temperature rose above 20 °C. Vaccinations of smolts against furunculosis and vibriosis before transporting them to the sea were then started, but in summer 1993 and 1994, when the seawater temperature rose to 23 °C, mortalities began to occur even among the vaccinates (5,9). The culture of this species has not been generally successful due to technical, ecological (high temperature of the Black Sea surface water) and marketing problems. Nevertheless, a few companies are still involved in Atlantic salmon farming in Turkey (8).

Aquaculture is growing rapidly worldwide, with fish being the primary source of animal protein in many countries. The stress and disease that accompany intensive fish culture have led to increased treatment with antibiotics and chemicals. Rapid detection and identification of fish pathogens is crucial to successful disease control. The identification of clinical and environmental Aeromonads has expanded considerably from the initial three major mesophilic species of *A. hydrophila*, *A. sobria*, and *A. caviae*, first isolated by Popoff (10). While the major rapid identification systems such as Vitek, API-20E, and API 20NE are beginning to recognize the significance of species identifications, it is reported that no single system encompasses an appropriate battery of tests to distinguish even these three species from one another without additional tests (11-15). One of the systems used in aquaculture for the rapid diagnosis of bacterial fish diseases is the API-20E as it is relatively cheap and simple to use. Although this system, as well as other manual systems, was initially designed for the identification of members of the family Enterobacteriaceae, it has now been adapted for identification of members of the family Vibrionaceae (11).

The aim of this study was to compare motile Aeromonas strains isolated from the intestinal flora of healthy salmon with those from salmon with motile Aeromonad septicemia found in Turkey during 1995 so that more detailed descriptions of pathogen bacteria and non-pathogen intestinal flora bacteria would become available.

Materials and Methods

Atlantic salmon (*S. salar*) were obtained from a fish farm with a capacity of 250 tons per year on the Black

Sea coast of Turkey in summer 1995. In total, 51 fish were sampled; 16 fish from freshwater (average weight: 75 g) and 35 fish from seawater (average weight: 2750 g). The mean freshwater and seawater temperature and dissolved oxygen at time of collection were 11 °C and 19 °C and 6 mg/L, respectively. The ventral surfaces of the fish were thoroughly scrubbed with an alcohol solution and standard aseptic procedures were used to make the biopsy. Samples from the intestinal tract of the healthy salmon were taken in these aseptic conditions and were diluted with alkali peptone water (16). After the homogenization of the samples, they were incubated in Tryptic soy agar (TSA) at 25-27 °C for 24 h. Identification was facilitated by examination of colonial morphology, as well as by examination of shape, arrangement, staining characteristics, motility, oxidase activity, oxidation-fermentation properties, and resistance to O/129 vibriostat of the cells. Oxidase-positive, Gram-negative, rod-shaped bacteria were cultured in OF medium to identify their carbohydrate metabolism. Indole, Voges-Proskauer, Methyl red, glucose fermentation, ornithine decarboxylase, motility and growth at 1-7% NaCl were checked by the standard tube method for species level identification. To compare with results obtained with standard biochemical tube tests, the API-20E system was used according to the manufacturer's instructions, by using bacteria as inocula suspended in 5 ml of 0.85% sterile saline. After 24 h incubation at 25-27 °C, reagents were added and the seven-digit profile number generated. The strains were stored on TSA slants at 4 °C and frozen at -80 °C with 15% glycerol (v/v) for long-term storage.

The incubation temperature used throughout this study was 25-27 °C and the readings of the tests in all the identification systems were performed after 48 h, but the standard tube reactions were examined for 7 days before being discarded as negative.

Results

On the basis of morphological, physiological and conventional biochemical tests, the Aeromonas strains isolated from fish were identified. Gram-negative, rod-shaped strains that were oxidase-positive, fermentative, motile and resistant to O/129 were identified at the species level with biochemical tests described by Popoff (10). Eight motile Aeromonas were isolated. Amongst

Table. Results of API 20E and standard tube tests of motile *Aeromonas* isolated from Atlantic salmon in the present study. Seven and eight were isolated from the Black Sea. F = Fermentative, R = Resistant.

	API 20E	Standard tube	API 20E	Standard tube	API 20E	Standard tube	API 20E	Standard tube	API 20E	Standard tube	API 20E	Standard tube	API 20E	Standard tube	API 20E	Standard tube
	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8
Gram	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cytochrome oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OF	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
O/129	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Growth at 37 °C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
Voges-Proskauer	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+	+
Methyl Red		-		+		-		-		-		-		-		-
Mannitol	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+
Gas from glucose		+		-		-		+		-		-		-		+
Acid from glucose	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+
Growth in 1% NaCl		+		+		+		+		+		+		+		+
Growth in 7% NaCl		+		+		+		+		+		+		+		+
Lysine Decarboxylase	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Arginine Dihydrolase	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
Ornithine Decarboxylase	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Esculin		+		+		+		+		+		-		-		+
Salicin fermentation		+		+		+		+		+		-		-		+
Ortho-nitro-phenyl-galactoside	+		+		+		+		+		-		+		+	
Sodium Citrate	+		+		+		-		-		-		+		+	
H ₂ S production	-		-		-		-		-		-		-		-	
Urease production	-		-		+		-		-		-		-		-	
Gelatinase	+		+		+		+		+		-		-		+	
Arabinose	+		+		+		+		+		-		-		+	
Sorbitol	+		+		+		+		+		-		-		+	
Tryptophane	-		-		-		-		-		-		-		-	
Rhamnose	+		+		+		+		+		-		-		+	
Melibiose	+		+		+		+		+		-		-		+	
Amygdalin	+		+		+		+		+		-		-		+	
NO ₂	+		+		+		+		-		-		-		+	
Lactose		-		-		-		-		-		-		-		-

1, 2, 3, 4, 5, 8: *A. caviae*

6, 7: *A. sobria*

these, two were identified as *A. sobria*. The other six strains were identified as *A. caviae*. The biochemical characteristics evaluated by using the conventional tube and API 20E system are listed in the Table.

Discussion

By the end of the examination of the 51 *S. salar* intestinal flora, eight motile Aeromonas were isolated. Amongst these, two were identified as *A. sobria* according to Austin and Austin (3), and Inglis et al. (2). All of these strains were negative for esculin, arginine, glucose, arabinose and fermentation of salicin. For the other six strains, all these characters were positive and they were assigned to the species *A. caviae*. Variable results between the API 20E system and conventional tube tests were detected in lysine decarboxylase and arginine dihydrolase. The other test results were similar for both conventional tests and the API 20E system.

Two Aeromonas strains were isolated from fish from the Black Sea. One was negative for esculin, arginine, glucose, arabinose and fermentation of salicin, and was therefore identified as *A. sobria*. The other was identified as *A. caviae*. The biochemical characteristics of *A. caviae* isolated from the Black Sea and from freshwater were very similar except for citrate, VP and gas production from glucose.

It was found that the API 20E system could be used to identify the Aeromonas strains under certain conditions, as shown by Toranzo et al. (17) and Burke et al. (18). However, it also became clear that API-20E strips could provide a tool for examining motile Aeromonas strains only if the API-20E profile index was not used and important biochemical reactions such as acid

production from glucose, Voges-Proskauer, esculin hydrolysis, and arginine dihydrolase were conducted simultaneously with the API-20E system to classify the Aeromonas isolates to species level. All the strains isolated in this study were evaluated without using the API 20E index in accordance with Toranzo et al. (12).

In this study, motile Aeromonas samples were isolated from healthy fish intestinal flora as in previous studies by various authors (1-4,16,19), thus demonstrating that Aeromonas are the main members of the normal intestinal flora of salmon in both freshwater and the Black Sea.

A. caviae was isolated from fish with the clinical signs of bacterial septicemia on the Black Sea coast of Turkey in 1991 and it has been reported as causing mortality rates up to 100% by Candan et al. (5). However, there were some differences between the isolates used by Candan et al. (5) and the isolates in this study, such as melibiose, rhamnase and fermentation of inositol. This indicated that the motile Aeromonas that can cause hemorrhagic septicemia can also be found in the intestinal flora of healthy fish cultured in the Black Sea environment. The conclusion of this study is, therefore, that these motile Aeromonas strains, as opportunistic pathogens, could cause infections under stress conditions such as high summer water temperatures as suggested by Popoff (10) and Groberg et al. (7).

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