

# Aeromonas Salmonicida In Californian Trout (Oncorhynchus Mykiss, Walbaum, 1792 ) And Some Biochemical Characteristics Of This Bacteria

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## Abstract

The aim was to determine if the bacteria *Aeromonas salmonicida* was the cause of the disease furunculosis, in Californian trout (*Oncorhynchus mykiss*, Walbaum 1792), as well as certain biochemical activities of the isolated strains.

Ten strains of the species *A. salmonicida* were isolated from samples taken from changed skin areas on fish raised in fisheries and all were immobile. The strains grow at a temperature of 22-23°C and colonies of them formed a brown pigment between the third and fifth days. The biochemical activities of the isolated strains were identical to the activities of the standard strain of *A. salmonicida*, which served as a control. In tendency of determination the presence of this bacteria in trout it has been done the rapid agglutination with the "O" antiserum against *A. salmonicida* which was a positive reaction. The isolated strains, multiplied in bacto-tryptose nutrient, produced a toxin that cause a cytopathogenic effect on pig kidney cell culture.

## INTRODUCTION

The taxonomy of genus *Aeromonas* is complex. Species *Aeromonas salmonicida* and *A. hydrophila* with related species *A. bestiarum* are included in the so-called 'A. hydrophila' complex (Janda and Abott, 1998). *A. salmonicida* is one of best-known representative from genera *Aeromonas*.

*Aeromonas salmonicida* is a gram negative, immobile, rod-shaped species of *Aeromonadaceae*, size 1.3 – 2.0 by 0.8 – 1.3 µm that grows at the temperature of 22-23°C, but never at 37°C (Inglis et al., 1993). It is oxidase positive, catalase positive, glucose-fermenting, facultative anaerobic species (Abbott et al., 2003). One of its main diagnostic characteristics is a production of soluble brownish pigment. Popoff (1984) suppose that the presence of pigment is not reliably diagnostic, because some variations between pigmented strains exist as well as in quantity of produced pigment as in a time of its appearing. *A. salmonicida* has the capability for generation of three different types of colonies. The most frequent are so called "petersham", smooth and intermeddling (G phase) colonies.

*Aeromonas salmonicida* is one of the most studied fish

pathogens, because of its widespread distribution, diverse host range and economically devastating impact on cultivated fish, particularly the salmonids. *A. salmonicida* has been recognized as a pathogen of fish for over 100 years (Abbott et al., 2003). It is described like an etiological agent of furunculosis in salmonids, some other its stripes causes eritrodermatitis in cyprinids, fester in salmonids and cyprinids, and some systematic infections in few worm water and sea water fishes. Furunculosis is established in Belgium, France, Switzerland, Austria, Germany, Great Britain, not only in fish raised in fisheries, but also in those living in open waters. The disease has spread from the continent of Europe to the United States and Canada, and has also been present in Australia since 1980 (Emmerich and Weibel, 1980).

Three major routes of infection have been suggested for *A. salmonicida*; the skin, the gills and the intestine (Inglis et al., 1993). The most thoroughly investigated sites have been the skin and the gills (Evelyn, 1996; Svendsen and Bøgwald, 1997) whereas the intestine as a route of infection has received less attention.

While *A. salmonicida* was traditionally thought of as a

pathogen of salmonids, global reports now confirm that this pathogen has been associated with clinical or covert disease in a variety of salmonid and non-salmonid species in freshwater, brackish water and sea water.

In addition to trout, *A. salmonicida* is present in some other fish species, such as, carp (*Cyprinus carpio*, L. 1758), perch (*Perca fluviatilis*, L. 1758), tench (*Tinca tinca*, L. 1758) and pike (*Esox lucius*, L. 1758).

*Aeromonas salmonicida* is a stable organism both in the sense of cultivation on nutritive media and regarding its physical and chemical characteristics (Griffin et al, 1953; Eddy, 1962; Ewing et al., 1961; Popoff, 1969; Donlon et al., 1983).

Ewing et al. (1961), and Liu (1961) present data on the antigenic characteristics of *A. salmonicida*. Their investigation established the “O” antigen and its relation to the “O” antigen of other bacteria, primarily *A. hydrophila*. It has been demonstrated that most strains of *A. salmonicida* produce pathogenic exogenous, which is protein composite and endogenous toxins, lyposacharide nature (Nomura and Saito, 1982; Atanackovic-Stojkovic, 1987; Asanin, 1990).

Cirkovic et al. (1998) working on problems of fish pathology on fisheries in Serbia show diversity of epizootiology situations according to sickness dispersion of different causes (viruses, bacteria, parasites and fungi). In their paper is presented fish diseases that, according to Federal Law for Animal Protection from diseases, endangers the whole country, and are very dangers for fish production and are obligate for registration and suppression. On the list of diseases is, between the others, and furunculosis.

Jeremic (1989), in her Ph.D. thesis, experimentalized with different vaccination methods against furunculosis in salmonids. The vaccine was prepared from field isolate from *A. salmonicida*. The different methods of fish vaccination contribute for good serological response and protect the vaccinated fish from expose pathogen field isolate of *A. salmonicida*.

In Serbia, activities in scientific area of fish bacteria are poorly, especially *A. salmonicida*. This is the main reason why we start with this kind of investigations in regard of their significance.

## **MATERIAL AND METHODS**

The material for this study originating from four salmonid

fisheries in Serbia, during two years. A total of 140 fish specimens of californian trout (*Oncorhynchus mykiss*, Walbaum, 1792) were examined. The fishes were 1400 to 1700 gr in weight, age from 18 – 19 months, and also some mature female with visible changes on skin.

Bacteriological examinations were performed on live and dead fishes with visible skin changes in the form of erosions or deeper necrotic lesions, which correspond to the expected alterations in trout furunculosis concerning appearance and shape.

The bacteria were isolated by sowing material from the mentioned skin areas and from organs (liver, spleen and kidneys) on two nutritive bacteriological media; bacto-tryptose agar and bacto-tryptose 5% blood agar. The treated media were incubated in a thermostat at 22-23°C and at 37°C. The samples were incubated for three days, and the growth on the medium surface was checked daily.

The characteristically formed colonies, round in shape with hemolysis and pigment, which multiplied at the temperature of 22-23°C, were isolated and resown on new media in order to obtain “clean” cultures for differentiation and typification. Typification was carried out on spaces bases, which were used for determining the biochemical activity of the isolated strains. All the isolated strains were examined by rapid serum agglutination on a plate, using diagnostic sera for rapid agglutination, namely an antiserum against *A. salmonicida* and an antiserum against *A. hydrophila*. We also prepared a standard strain, which served as a control and with it were compared the characteristics of the isolated strains. Only those isolates which had positive agglutination with antiserum to *A. salmonicida* were further processed and declared isolates of *A. salmonicida*.

The pathogenicity of the isolated strains was checked on pig kidney tissue culture, by introducing sterile filtrates and observing the appearance of cytopathogenic effects in culture.

## **RESULTS AND DISCUSSION**

Ten strains of *A. salmonicida* were isolated on the basis of bacteriological examinations of samples taken from 140 diseased fish. All the isolated strains were examined biochemically and, on the grounds of the results obtained as well as positive agglutination with antiserum against *A. salmonicida* and negative agglutination with antiserum against *A. hydrophila*, it was confirmed that they belonged

to the species *A. salmonicida*. The characteristics of the isolated strains were compared to those of a standard strain, which served as a control.

The specific characteristics of the isolated strains of *Aeromonas salmonicida* are presented in Table 1.

**Figure 1**

Table 1: Biochemical activities of the isolated strains of

Type of test	Mark of strain and achieved reaction										Standard strain
	5	11	26	29	37	54	71	82	97	117	
H <sub>2</sub> S	-	-	-	-	-	-	-	-	-	-	-
Nitrates	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+
V.P.	-	-	-	-	-	-	-	-	-	-	-
Indol	-	-	-	-	-	-	-	-	-	-	-
Esculin	+	+	+	+	+	+	+	+	+	+	+
Hemolysis	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+
Manitol	+	+	+	+	+	+	+	+	+	+	+
Saccharose	-	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-	-
Growth 22-23°C	+	+	+	+	+	+	+	+	+	+	+
Growth 35-37°C	-	-	-	-	-	-	-	-	-	-	-

The isolated strains were found to have the following characteristics: no H<sub>2</sub>S production in media; nitrates were positive; indol was negative; catalase was positive; V.P. was negative; esculin was positive. There was a zone of hemolysis around the grown colonies. Gelatin, glucose, arabinose and manitol were degraded but not saccharose. All isolated bacteria strains were immobile. The examined strains multiplied at a temperature of 22-23°C, while there was no growth at 37°C. The colonies of the examined strains produced a brown pigment after 3 to 5 days.

The results of agglutination reactions with anti “O” antisera to *A. salmonicida* and antigen of the isolated strains and control antigen are presented in Table 2.

**Figure 2**

Table 2: The results of rapid agglutination with O” antiserum against and

Type of antiserum	Mark of antiserum (examined strain)										<i>A. salmonicida</i>	<i>A. hydrophila</i>
	5	11	26	29	37	54	71	82	97	117		
<i>A. salmonicida</i>	+	+	+	+	+	+	+	+	+	+	+	-
<i>A. hydrophila</i>	-	-	-	-	-	-	-	-	-	-	-	+

The isolated strains of *A. salmonicida*, originating from different samples taken from diseased places on trout skins, gave a positive reaction for agglutination with the “O” antiserum against *A. salmonicida*, while the agglutination reaction with the “O” antiserum against *A. hydrophila* was negative.

All the isolated strains produced a toxin in the filtrate of the nutrient culture of the multiplied bacteria and its presence

was confirmed by the appearance of a cytopathogenic effect on pig kidney tissue culture. Cytotoxins and enter toxins (including those with hemolytic activity) are the most important for their pathogenicity (Merino et al., 1992, 1995).

The results obtained in this work clearly demonstrate that the cause of furunculosis in trout is present in fisheries of Serbia and that *Aeromonas salmonicida* can be detected by bacteriological examination of material taken from diseased areas and using serological examination.

The biochemical activities of the isolated bacteria strains in our fisheries indicate that the isolated agent is very similar to that described by Griffin et al. (1953); Ewing et al. (1961); Popoff (1969) and Donlon et al. (1983).

The reactions of positive agglutination with the antiserum to the “O” antigen of the standard strain of *A. salmonicida* confirmed the presence of the pathogen and ruled out the possibility of the presence of *A. hydrophila* as the cause of the disease. It has been demonstrated in our investigation that the detection of endogenous and exogenous toxins in the culture filtrate nutrient of the isolated bacteria strains by their cytopathogenic effect on pig kidney cell culture, can be a reliable indicator for confirming the cause of the disease. Similar data have been presented by Nomura and Saito (1982) and Atanackovic-Stojkovic (1987).

## CONCLUSIONS

The results from this study indicate to validity of beginning the research of *A. salmonicida* presence in Serbian fisheries. In our further intensive investigation we will attempt to get complete picture about presence of bacteria and their dispersion in fisheries which can be of multiplied use for our scientific audience, fishing and its development. The aim of this is protection and indication of furunculosis and other diseases in *Oncorhynchus mykiss* and other salmonids.

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