Acta Veterinaria (Beograd), Vol. 48. No. 4, 217–224, 1998.

UDK 619:579.843.2:639.211.3

SOME BIOCHEMICAL CHARACTERISTICS AND THE OCCURRENCE DEGREE OF PRESENCE OF AEROMONAS SALMONICIDA IN CALIFORNIAN TROUT (ONCORHYNCHUS MYKISS)

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(Received, 5, April, 1998)

The objective of this work was to determine the abundance of the bacterum Aeromonas salmonicida, the cause of the disease furunculosis, in Californian trout (Oncorhynchus mykiss), as well as certain biochemical activities of the isolated strains.

Ten strains of the species Aeromonas salmonicida were isolated from samples taken from changed skin areas on fish raised in fisheries. Two strains were isolated in Fishery I, one strain in Fishery II, four strains in Fishery III, and three strains in Fishery IV. The cause of the disease was not found in samples of internal organs of the diseased fish.

The isolated strains of bacteria had the following biochemical activities in the respective media: H_2S production was not established; nitrates were positive; catalase was positive; V. P. and indol negative, esculin positive, hemolysis positive; there was degradation of gelatin, as well as of glucose, arabinose, and manitol. Saccharose degradation was not found. All bacteria strains were immobile. The strains grew at a temperature of 22-23^{0}C, and did not grow at 35-37^{0}C. Colonies of the examined strains 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 Aeromonas salmonicida which served as a control.

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

The isolated strains, multiplied in bactro-tryptose nutrient, produced a toxin whose presence was confirmed by the appearance of a cytopathogenic effect on pig kidney cell culture.

Key words: Californian trout, Aeromonas salmonicida, biochemical activities, toxin.

INTRODUCTION

Aeromonas salmonicida is one of the first described causes of diseases in fish. Thus Emmerich and Weibel (1984) isolated the agent causing a contagious disease in trout from diseased and dead stream trout (Salmo trutta m. fario), originating from a spawning ground in Austria. The agent is today known as Aeromonas salmonicida und the disease it causes is called furunculosis.

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.

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

The agent causing furunculosis in trout, Aeromonas salmonicida, har also been known as: Bacterium salmonicida (1984), Bacterium devorans (1896), Bacterium salmonicida (1897), Bacterium trute (1902). According to Berge(1957), the bacterium was named Aeromonas salmonicida and placed in the genus Aeromonada, family Vibrionaceae.

Aeromonas salmonicida is a stable organism both in the sense of cultivation on nutritive media and regarding its physical and chemical characteristics (Grifin, 1953a; Eddy, 1960; Ewing, 1961; Popoff, 1969; Donlon, 1983). It is an immobile, gram - negative rod, which grows at the temperature of 22 - 23^{0}C, but never at 37^{0}C. It produces catalas and oxidase. Pigmented colonies grow in same media, which is largely determined by their composition (Griffin, 1953b). This characteristic is important for their identification although one should have in mind that there are also achromus strains of Aeromonas salmonicida.

Ewing (1961), and Liu (1961) present data on the antigenic characteristics of Aeromonas salmonicida. Their investigation established the "O" antigen and its relation to the "O" antigen of other bacteria, primarily Aeromonas hydrophila. Views are divided regarding antigenic similarity with other bacteria. Some believe there are no cross-reactions between the antigens of one and the other, while other have shown cross reactions in agglutination, which indicates that the antigens are related.

It has been demonstrated that most strains of Aeromonas salmonicida produce pathogenic exogenous and endogenous toxin, (Nomura, 1982; Atanacković, 1987; Ašanin, 1990).

MATERIAL AND METHODS

Bacteriological examinations were performed on live and dead fish 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. A total of 120 fish were examined, originating from four trout fisheries. The bacteria were isolated by sowing material from the mentioned skin areas and from organs (the 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^{0}C and at 37^{0}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^{0}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 agains Aeromonas salmonicida and an antiserum against Aeromonas hydrophila.

Only those isolates which had positive agglutination with antiserum to Aeromonas salmonicida were further processed and declared isolates of Aeromonas 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 Aeromonas salmonicida were isolated on the basis of bacteriological examinations of samples taken from 120 diseased fish originating from four trout fisheries. All the isolated strains were examined biochemically and, on the grounds of the results obtained as well as positive agglutination with antiserum against Aeromonas salmonicida and negative agglutination with antiserum against Aeromonas hydrophila, it was confirmed that they belonged to the species Aeromonas 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.

Table 1. Biochemical activities of the isolated strains of A. salmonicida.

Type of test	Mark of strain and achieved reaction										Standard strain
	5	11	26	29	37	54	71	82	97	117	
H_2S	–	–	–	–	–	–	–	–	–	–	–
Indol	–	–	–	–	–	–	–	–	–	–	–
Catalase	+	+	+	+	+	+	+	+	+	+	+
Nitrates	+	+	+	+	+	+	+	+	+	+	+
V. P.	–	–	–	–	–	–	–	–	–	–	–
Glucose	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+
Manitol	+	+	+	+	+	+	+	+	+	+	+
Saccharose	–	–	–	–	–	–	–	–	–	–	–
Esculin	+	+	+	+	+	+	+	+	+	+	+
Hemolysis	+	+	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+	+	+
Motility	–	–	–	–	–	–	–	–	–	–	–
Growth 22–23°C	+	+	+	+	+	+	+	+	+	+	+
Growth 35–37°C	–	–	–	–	–	–	–	–	–	–	–

The isolated strains were found to have the following characteristics: no H_2S 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^{0}C, while there was no growth at 37^{0}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 Aeromonas salmonicida and antigen of the isolated strains and control antigen are presented in Table 2.

Table 2. The results of cross agglutination with antiserum against A. salmonicida and A. hydrophila

Type of antiserum	Mark of antiserum (examined strain)										A. salmonicida	A. hydrophila
	5	11	26	29	37	54	71	82	97	117		
A. salmonicida	+	+	+	+	+	+	+	+	+	+	+	–
A. hydrophila	–	–	–	–	–	–	–	–	–	–	–	+

It can be seen that all the isolated strains showed an agglutination reaction with the "O" antiserum of Aeromonas salmonicida and that there was a negative reaction for agglutination with the "O" antiserum of Aeromonas hydrophila.

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. (Figure 1.)

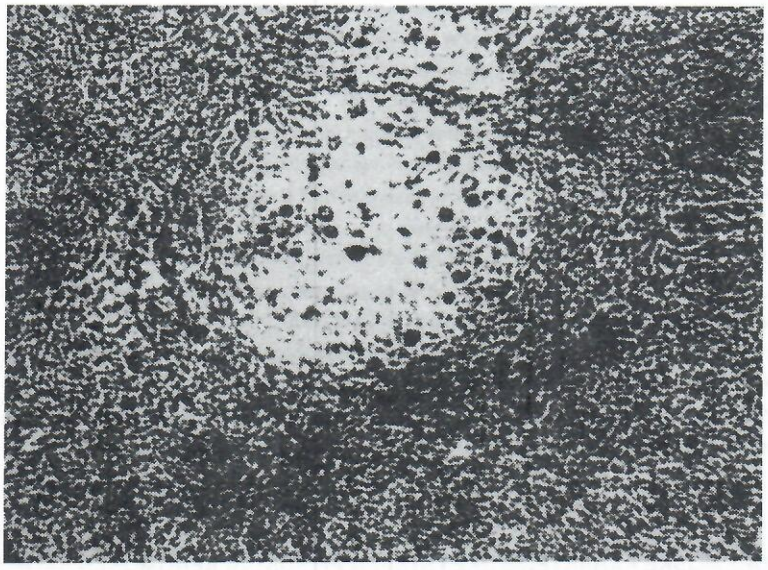

Figure 1. Tissue culture inoculated with Aeromonas salmonicida toxin after 48h

The results obtained in this work clearly demonstrate that the cause of furunculosis in trout is present in fisheries 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 (1953)a; Ewing (1961); Popoff (1969) and Donlon (1983).

The reactions of positive agglutination with the antiserum to the "O" antigen of the standard strain of Aeromonas salmonicida confirmed the presence of the pathogen and ruled out the possibility of the presence of Aeromonas hydrophila as the cause 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 (1982) and Atanacković (1990).

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NEKE BIOHEMIJSKE KARAKTERISTIKE I STEPEN PRISUSTVA AEROMONAS SALMONICIDA KOD KALIFORNIJSKE PASTRMKE (ONCORHYNCHUS MYKISS)

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SADRŽAJ

Cilj ovog rada je bilo utvrđivanje stepena prisustva bakterije Aeromonas salmonicida, uzročnika oboljenja furunkuloze pastrmki i određivanje nekih biohemijskih aktivnosti izolovanih sojeva.

Bakteriološkim pregledom 120 uzoraka riba sa četiri ribnjaka za uzgoj pastrmki, izolovano je 10 sojeva bakterije Aeromonas salmonicida. Sojevi su izolovani sa promenjenih mesta na koži, dok uzročnik nije ustanovljen iz unutrašnjih organa riba.

Dijagnostički materijal uzet od riba, bakteriološki je obrađen, tako što je zasejan na hranljive bakteriološke podloge: triptoza soja agar i triptoza soja 5% krvni agar. Inkubiranje podloga je vršeno na temperaturi od 22 C do 23C i 37 C. Ispitivani uzročnik nije rastao na temperaturi od 37 C. Posle tri do pet dana inkubiranja, izrasle kolonije su proizvele smeđi pigment.

Karakteristične kolonije koje su se odlikovale okruglim izgledom, sa zonom hemolize na krvnom agaru i pojavom pigmenta, prenete su na nove podloge radi dobijanja "čistih" kultura u cilju daljeg postupka determinisanja.

Svi izolovani sojevi su bili ispitani radi određivanja biohemijske aktivnosti bakterija. Utvrđene su najznačajnije osobine: izostanak stvaranje H_2S i indola, katalaza i nitrati su bili pozitivni, VP negativan, razlagali su glukozu, arabinozu i manitol. Saharoza je bila negativna. Eskulin i hemoloza su bili pozitivni. Izolovani sojevi razlagali su želatinozu. Svi sojevi su bili nepokretni.

Ispitani sojevi su metodom brze serumske aglutinacije sa anti serumom protiv Aeromonas salmonicida dali pozitivnu aglutinaciju.

Filtrat dobijen razmnožavanjem izolovanih sojeva na triptoza soja bujonu i inokulisan u kulturu tkiva prasećeg bubrega, dao je citopatogeni efekat posle 48 časova inkubiranja na temperaturi od 37C.

Na osnovu ukupnih rezultata ispitanih sojeva koji su izolovani iz promenjenih mesta na koži riba, ustanovljeno je da pripadaju vrsti bakterije Aeromonas salmonicida koja je uzročnik furunkuloze riba.