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CULTIVATION OF SYSTEMIC "MYXOBACTERIUM" FROM RAINBOW TROUT

By A. VUILLAUME[1], E. SOCHON[2] & R. LESEL[3]

In 1986, a bacterial agent associated with a disease in rainbow fingerlings, was detected in a few hatcheries located in South-West France. Mortalities could reach 80%.

The disease was observed in fingerlings (0,7 to 10 g) in water with a temperature ranging from 5 to 15°C. Affected fish generally presented bilateral exophthalmia, abdominal swelling, dark appearance, listlessness and anorexia.

Externally, gills were anaemic with haemorrhages in the lamellae. Internally, liver and kidney were discoloured, the kidney hypertrophic, the gut distended with acute enteritis. Most noticeable was splenomegaly (enlarged to 10 times normal volume) with necrotic zones or completely necrosed.

Petechial haemorrhages were present in the adipose tissues.

BACTERIOLOGY

May Grunwald Giemsa stained blood, kidney and particularly spleen smears revealed groups of thin round-ended bacteria (3-6 u x 0,75 u). These bacteria were free but also very abundant within macrophages; a few bacteria were observed inside vacuoles. The number of intracellular bacteria varied between cells and these filled with bacteria had an ecentric and pyknotic nucleus. This suggests that the bacteria were capable of destroying the macrophage system.

CULTURE

Isolation medium for myxobacteria (ORDAL and ANACKER agar) and also other commonly used media to detect fastidious organisms (chocolate agar: PASTEUR 63934; Columbia sheep blood agar: PASTEUR 63954) did not permit growth of the organisms. Growth was obtained only on egg-enriched COLETSOS agar + ossein (PASTEUR 53155 and 53165: Institut Pasteur Production, 36 rue du Docteur Roux 75725 Paris Cedex 15), generally used for isolation of mycobacteria.

Spleen samples were spread with a loop over the agar and incubated at 20°C. After 48 hours, colonies developed which were regular, rounded, yellow-orange, 0.25-0.5 mm diameter, smooth, with bright mucous slightly convexed surface. Colonies tended to spread along each side of the seeding line. They were very compact and difficult to spread on a slide. Direct microscopic examination of living cells revealed a sliding motility.

Some physiological and biochemical characteristics of these bacteria are reported in Table 1. Antibiosensitivity assays were carried out on enriched COLETSOS agar + ossein (Table 2).

It will be of interest to compare these bacteria with myxobacteria observed by Roberts (1988), which caused heavy mortalities in young marine farm fish, salmon in particular. In both cases there was "a very obvious and generalised invasive myxobacterial infection involving the growth of the bacteria in all organs".

Table 1 - Some physical and biochemical characteristics of the bacterial isolate.

Growth at:	10°C	+
	20°C	+
	37°C	-
	Gram	-
	Oxidase	-
	Catalase	-
	Amylase	-
	Tween-esterase	-
	Proteolysis	+

Table 2 - Sensitivity to antibiotics

Antibiotics	Diam. Disc mm	Antibiotic ug/disc	Sensitivity
Flumequin (1)	6	30	+
Oxolinic acid (2)	6	10	-
Oxytetracyclin (1)	6	30	+
Chloramphenicol (1)	6	30	+
Doxycyclin (1)	6	30	+
Nitrofurantoïn (1)	6	300	+
Trimethoprim (1) + Sulfametoxazol (1)	6	1,25 / 23,75	+

(1) BioMérieux
Marcy-l'Etoile
69752 Charbonnières-les-Bains
Cedex (France)

(2) Institut Pasteur Production
36, rue du Docteur Roux
75725 Paris Cedex 15

Reference
Roberts R.J. (1988). Aquaculture and slime bacteria.
Fish Farming Int., 15, p. 23

Author's address:
(1) Directeur du laboratoire des Services Vétérinaires des Landes, 120, Avenue Pasteur, 40000 Mont-de-Marsan (France).
(2) FRGDS, section Aquacole, 40000 Mont-de-Marsan (France)
(3) INRA Station d'Hydrobiologie de Saint Pée/Nivelle B.P.3 64310 Ascain (France)