

RAINBOW TROUT MORTALITIES ASSOCIATED WITH A MIXED INFECTION WITH *Citrobacter freundii* AND IPN VIRUS

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Citrobacter freundii has not been widely recognised as a fish pathogen except in some references (Sato *et al.*, 1982; Conroy, 1986). However during the last two years, the number of isolations of *Citrobacter freundii* from diseased fish has increased in USA and Spain (Baya *et al.*, 1990).

Usually the isolation of the bacteria was associated with high levels of pollution. However, in the present case, a high mortality occurred in April, 1991 in rainbow trout (2-5 g. weight) on a Spanish fish farm using 14°C spring water with apparent low levels of pollution.

Bacteriological samples of moribund fish were taken from liver and kidney, and streaked on tryptic soy agar (TSA) (Biomerieux) for primary isolation of bacteria. The plates were incubated at 24°C for one day and a large fast growth of colonies was produced. The growth in TSA medium supplied with 5% lamb blood (Biomerieux) was slower than in TSA simple medium. At the same time, a virological analysis was performed on the largest affected trout (4-5 g. weight). Cell lines used were the BF-2 (buegill fibroblast) and EPC (epithelioma papillosum carpio). A cytopathic effect appeared on the 8th day of infection. The virus was identified as IPN virus, which was isolated in the same farm two months before.

Some characteristics of *C. freundii* isolated were: rods, Gram negative, motility by flagella, catalase positive, cytochrome oxidase negative and the biochemical patterns of the bacteria are similar to the ones de-

scribed by Baya *et al.*, 1990. They are positive in 9 tests using the API 20 E system, (API System S.A. La Balme. Les Grottes, France), (ONPG, HS, GLU, MAN, SOR, MEL, RHA, ARA and AMY) and negative in 11 other reactions (ADH, LDC, CIT, ODC, URE, TDA, IND, GEL, VP, INO and SAC). The drug sensitivity test is described in Fig. 1.

The importance of *C. freundii* as a pathogenic agent, alone or associated with other fish health problems (pollution, viruses or bacterial diseases) appears to be increasing.

Summary

Citrobacter freundii has been isolated from farmed spanish rainbow trout (*Oncorhynchus mykiss*). This article describes its isolation with the presence of IPNV in the farm.

The biochemical patterns of the bacteria are similar to ones described by other authors.

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References

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Fig. 1.-Drug sensitivity of *C. freundii* (- resistant, + low sensitivity, ++ sensitive).

Lincomycin	-	Oxolinic acid	++
Spiramycin	-	Oxytetracyclin	-
Furazolidone	+	Flumequine	++
Ampicillin	-	Kanamycin	-
Erythromycin	-	Chloramphenicol	-
Sulfonamide	-	Amoxicillin	+
Gentamycin	++	Nitrofurantoin	+
Streptomycin	+	Penicillin	-