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HOMOZYGOUS RAINBOW TROUT CLONES AS A TOOL TO SCREEN THE VIRULENCE PROPERTIES OF *FLAVOBACTERIUM PSYCHROPHILUM*

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Introduction

Flavobacterium psychrophilum infection is an important concern for European freshwater trout farmers. Although the disease has been known for a long time in North America and long years of investigations have been devoted to the responsible agent, studies were often thwarted by the fastidious growth of the bacterium, its low thermal preferences and its poorly documented metabolic requirements. In spite of several attempts having resulted in the description of standardized procedures to prepare viable *F. psychrophilum* cultures and produce the disease experimentally, different kinds of difficulties are still commonly encountered. For instance, self-agglutination may be expressed more or less regularly, generally associated to adherence properties to glass and plastic; the slow growth at optimal temperatures of 15 to 18°C increases the chances of contamination with metabolically more active environmental microorganisms; the choice and preparation of culture media ensuring regular and reproducible yield of bacteria with acceptable viability are also important, any change or fortuitous event being likely to induce variations in the cell survival and culturability.

The application of endomitotic gynogenesis to produce homozygous rainbow trout (*Oncorhynchus mykiss*) clones differing in their susceptibility to flavobacteriosis has recently opened new possibilities for thorough study of virulence determinants and analysis of the host-pathogen relationships.

Materials and methods

Several experiments were first conducted to assess susceptibility differences to cold water disease among rainbow trout homozygous clones. Two *F. psychrophilum* strains originally isolated from rainbow trout (JIP 02-86) and coho salmon *O. kisutch* (THC 02-90) were used. Injection challenges were performed at 10 °C on 12 different clones and on the control trout strain "Sy" from which the clones had been derived. Bacterial suspensions were produced according to standard procedures (Garcia *et al*. 2000) and administered intra-muscularly (IM) at different doses to groups of 40 individuals per group and per dose. All fish injected with the same strain and the same dose were accommodated together into a single tank and kept under observation for 30 days. Their genetic origin was redrawn using microsatellite markers at the end of the experiment, after dead fish had been collected daily and all survivors sacrificed. Survival curves were then plotted. External lesions were also appreciated and recorded when present at the inoculation site. Some trials using immersion challenge were also performed with strain JIP 02-80. In a second step and in parallel to these *in vivo* experiments, preliminary insight in the susceptibility of the virulent *F. psychrophilum* isolates to phagocytosis and bactericidal properties of the trout serum was also initiated using clones with contrasted survival after challenge with the JIP 02-86 strain.

Results and discussion

Marked differences were observed in the susceptibility of the trout clones and were pretty much confirmed according the doses and challenges. Overall, the differences after IM injection could range from 0 to 100 % survival, depending on the clone, strain an dose. The rainbow trout isolate JIP 02-86 constantly proved more virulent for trout than

THC 02-90. In a few cases, conflicting results could occur in a same clone with different bacterial isolates. Such variations could reveal casual differences among clones in the defence mechanisms opposed to the infection and their efficacy. Likewise, an interesting contrast was noticed between mortality and clinical signs observed respectively with JIP 02-86 and THC 02-90. Infection with JIP 02-86 appeared more typical, with mortality starting after a period of 8-10 days and progressing regularly for 3 weeks. In the case of THC 02-90 high doses of bacteria were required to produce an earlier and shorter clinical outbreak which lasted only a few days and ceased suddenly without reaching very high mortality rate. In that last case, the curve appearance suggests a toxin exposition rather than a classical infection course resulting in progressive tissular invasion. Actually, active necrosis of muscular tissues resulting in deep cavities at the site of infection was observed with JIP 02-86 only. It so appeared that the virulent properties of *F. psychrophilum* may be founded on different mechanisms depending on the isolate.

Waterborne infection may prove effective when highly virulent isolates are used, the working dose being generally adjusted around 10^7 cfu /ml. Although this requirement was not always verified in the tests conducted on selected trout clones, survival differences were observed again. Correlation with IM infection results was more questionable, what might be attributed to differences expressed by some clones between mucosal ans systemic immune mechanisms.

The bactericidal effect of serum was observed in one out of two clones tested, with heated serum only, a rather surprising result which will have to be confirmed on every available clones.

Conclusions

To summarize, experimental disease performed in homozygous trout clones with a range of susceptibility allows to suspect different mechanisms in the pathogenicity of JIP 02-86 and THC 02-90 isolates and, correlatively, differences in the defence mechanisms likely to be opposed to the infection by different individuals. Beside the method refinement represented by homozygous fish use in experimental disease models, it is anticipated that the variations observed between immersion and parenteral infection in fish of the same genetic origin may be of significance for exploring host-pathogen relationships.

At last, complementary investigations in bacterial virulence should be usefully guided by clinical observation collected from fish experimentation, inasmuch as some virulence properties in *F. psychrophilum* may differ from those of other pathogenic Gram-negative bacteria (as suggested when testing serum bactericidal effect) and may display unexpected variations according to bacterial strains, fish populations and environmental conditions. The study of this bacterium appears a rather difficult challenge which will certainly have to be taken up through enlarged approaches and multidisciplinary programmes in which not only functional studies but genomic studies, epidemiological data and typing of virulent isolates (Nicolas *et al*. 2008) will also be of importance.

Reference

Garcia C., Pozet F., Michel C., 2000. Standardization of experimental infection with *Flavobacterium psychrophilum*, the agent of rainbow trout *Oncorhynchus mykiss* fry syndrome. *Dis. Aquat. Org.*, 42: 191-197.

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