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EPIDEMIOLOGY OF THE SALMONID FISH PATHOGEN *FLAVOBACTERIUM PSYCHROPHILUM* : OUTCOME OF MOLECULAR INVESTIGATIONS

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Flavobacterium psychrophilum est l'agent d'une septicémie hémorragique chez les salmonidés vivant en eau douce. La maladie, connue à l'origine en Amérique du Nord uniquement, est ensuite apparue dans différents pays Européens et plus récemment au Japon, en Tasmanie et au Chili. Elle provoque des mortalités importantes chez les alevins de salmonidés, mais aussi différentes formes chroniques. Dans le but de mieux connaître l'épidémiologie de la maladie, une collection importante de souches de F. psychrophilum, ayant différentes origines géographiques et isolées chez diverses espèces de poissons, a été étudiée en utilisant 3 méthodes de typage moléculaire : l'amplification en chaîne aléatoire de l'ADN (RAPD), les profils plasmidiques et la ribotypie. Il ressort de cette étude qu'il n'existe pas de corrélation entre les profils RAPD et l'origine géographique des différentes souches, mais certains profils sont clairement associés à l'espèce de poisson de laquelle elles ont été isolées. Une autre amorce donnant le même profil pour toutes les souches de F. psychrophilum permet de la différencier des espèces voisines coexistant dans le même environnement et souvent isolées chez les poissons. L'étude des plasmides montre que 71 % des souches (43/60) ont au moins un plasmide. La présence d'un plasmide majoritaire de taille estimée à 3,4 Kb chez 28 (46 %) souches a été établie. L'étude des profils de restriction des gènes d'ARN ribosomaux (ribotypie) montre que cette méthode est plus discriminante que les 2 précédentes. La combinaison des résultats obtenus par ces 3 méthodes montre leur capacité à fournir des marqueurs épidémiologiques importants pour le suivi de la maladie.

INTRODUCTION

Flavobacterium psychrophilum(Bernardet et al., 1996) (syn. *Cytophaga psychrophila, Flexibacter psychrophilus*) is the agent of cold-water disease and rainbow trout fry syndrome in salmonid fish. Originally isolated in North America only, it also causes now severe mortalities in many salmonid hatcheries in several European countries, Japan, Chile and Tasmania. Several other *Flavobacterium* species may be isolated from diseased freshwater fish. Some of them (i.e. *Flavobacterium columnare, F. branchiophilum, F. johnsoniae*) have been demonstrated to be pathogenic, at least in certain circumstances, while the pathogenicity of other species [*F. hydatis* (syn. *Cytophaga aquatilis*), *F. succinicans*] has not been established.

In order to unravel the epidemiology of the disease, a collection of 60 *F. psychrophilum* strains representing many geographic origins and fish hosts has been investigated by using 3 different molecular methods : random amplified polymorphic DNA (RAPD) (Williams et al., 1990), plasmid profiles, and rRNA gene restriction patterns (ribotypes). These techniques, used alone or in various combinations, may generate interesting epidemiologic markers.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Sixty strains isolated from different geographic areas (i.e. several European countries, USA, Japan and Tasmania) over the years were included in this study. All *F. psychrophilum* strains were grown on Anacker and Ordal agar (Anacker & Ordal, 1955) supplemented with 0.45 % tryptone. Other valid *Flavobacterium* species were grown on the same medium at 22°C.

Preparation of DNA

DNA of each strain was extracxted as described by Chakroun et al. (1997).

RAPD

Amplification, electrophoresis conditions and analysis of profiles were performed as described by Chakroun et al. (1997).

Plasmid profiles

Plasmid profiles were performed according to Takahashi & Nagano (1984).

Ribotyping

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Ribotypes were obtained using the new method developed by Regnault et al. (1997). After screening of 20 different restriction endonucleases, *Hinc* II and *Pvu* II were selected for further analysis of all strains.

Computer-assisted analysis of the DNA fingerprints
Each photo was imaged with a laser densitometer (Apple Computers, Cupertino, CA), and the DNA fingerprints were normalized and analyzed by using the TAXOTRON package (Institut Pasteur, Paris, France). The densitometric traces were analyzed using the single linkage method.

RESULTS

RAPD

Two primers (OPH06, OPH08) provided different patterns among the strains of *F. psychrophilum*, while another primer (OPG10) differentiated *F. psychrophilum* from the 9 other valid *Flavobacterium* species. Twenty-three rainbow trout (*Oncorhynchus mykiss*) isolates among 25 shared the same combined (OPH06+OPH08) profile, while 2 isolates exhibited another profile. The 16 coho salmon (*Oncorhynchus kisutch*) isolates were separated into 2 clusters, each exhibiting a different combined pattern and grouping both American and Japanese strains. The 4 Japanese isolates from ayu (*Plecoclossus altivelis*) shared the same very distinctive combined pattern. This was also the case for the 5 Australian strains isolated from Atlantic salmon (*Salmo salar*). The strains isolated from non-salmonid fish species occupied isolated positions on the dendogram.

Plasmide

Several plasmid profiles occurred among the strains. Most of the strains (43/60) exhibited at least one plasmid. Twenty-eight strains shared the same plasmid, which relative molecular weight was 3,4 Kb.

Ribotyping

More than 40 different profiles were generated when the data obtained with the 2 endonucleases were combined and analyzed, but computer analysis revealed an overall high similarity among these profiles.

DISCUSSION

RAPD provided a rapid and clear differentiation of *F. psychrophilum* from related bacterial species as well as an intra-specific typing of *F. psychrophilum* strains depending on the primer used. No correlation occurred between RAPD patterns and the geographic origin of the strains, but there was a clear association between some profiles and the species of fish form which the strains were isolated. This was particularly obvious in the case of the rainbow trout isolates, as most of them exhibited the same profile independant of their geographic origin. No correlation was noticed between plasmid profiles and either geographic origin or fish host, but they provided further differentiation among strains exhibiting the same RAPD profile. Ribotyping proved more discriminant than the two other methods, yielding a larger number of different profiles, but the data proved more difficult to interpret. However, combination of ribotypes with data generated by the other techniques may prove usefull for an efficient typing of *F. psychrophilum* strains and a powerfull epidemiologic tracing.

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