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Traditional and Phylogenetic Approaches in the Diagnosis and Identification of Pathogens in Mariculture

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Abstract

Traditionally, the most common approach to diagnosis of microbial fish pathogens has relied on *in vitro* isolation of the microorganism and the information provided by its phenotypic features. However, viruses are generally highly species-specific and established cell lines do not necessarily show cytopathic effect, many species of bacteria are difficult or impossible to culture *in vitro*, while parasitic microorganisms often have a complex life cycle that requires propagation in live hosts. An increasing number of microbial pathogens are identified today by molecular methods, without the need for isolation. A PCR direct method for detection and identification of *Mycobacterium marinum* based on the 16S rRNA gene sequence was successfully developed already 13 years ago at NCM. Comparison of the 16S rRNA sequence of *Streptococcus iniae* isolates revealed that, despite phenotypic, biochemical and pathogenetic similarities, marine and freshwater isolates were different strains. With time, however, it has become clear that 16S rRNA gene sequences alone are often insufficient to detect variation within bacterial species, and today other specific loci are also being employed. More recently, on the basis of *hsp*65 gene in addition to 16S rRNA gene, Israeli *M. marinum* isolates in marine and freshwater fish were found to belong to two distinct strains, and both were different from Israeli *M. marinum* clinical (human) isolates. Specific 18S rDNA probes for detection of elusive life stages of two myxosporean parasites, *Kudoa iwatai* and *Enteromyxum leei*, in sea bream and sea bass are being employed in studies conducted over the last few years at NCM. By using whole-genome structures rather than single gene sequences, two fingerprinting techniques - Amplified Fragment Length Polymorphism (AFLP) and Randomly Amplified Polymorphic DNA (RAPD) - have provided a generally higher level of precision in genotyping. However, while the AFLP method revealed broad polymorphism among *S. iniae* isolates, the RAPD method did not provide additional information. These examples show that not all regions of the DNA are equally useful in diagnosis and genotyping and therefore there is no single “best” molecular method. Molecular strategies have provided a phylogenetic approach to determining identification and taxonomic position by grouping closely related organisms that share a relatively recent ancestry into clusters. Although the question remains of how much genetic diversity is permissible in a discrete cluster for its members to be regarded as a single taxon, the ability to place a microorganism in a given taxon on the basis of its evolutionary development is of importance: if known members of the same family do not have a “clean bill” concerning their pathogenicity, any related organism may be justifiably regarded as a potential offender. Traditional methods and molecular methods provide different levels of information: only their combination offers a comprehensive insight into the microorganism’s nature.

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