Genetic characterisation of Brachionus sp. clones using the 16SrDNA marker with the SSCP (Single Strand Conformation Polymorphism) technique and sequencing

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Introduction

The development of the mass production of high quality fingerlings of marine fish species in Europe was made possible by improvements in the techniques for producing and utilizing live food: rotifers Brachionus plicatilis and Brachionus rotundiformis (Sorgeloos & Sweetman, 1993; Planas & Cunha, 1999, Dhert et al., 1993; Lavens et al., 1994; Dehaesque et al., 1995; Sorgeloos et al., 1996). The rotifer production is still the biggest problem for the fingering production: the mass culture of these rotifers is very unpredictable (Candreva et al., 1994). Periods with total mortality or reduced reproduction are quite frequently occurring. Does the controlled mass culture of rotifers leads to an impoverishment in the genetic diversity of the cultured rotifers and does this fact in its turn make the rotifer culture more susceptible to crashes if there is a change in the biotic or abiotic conditions prevailing in the culture?

The genetic approach to the project aims at two different goals: 1) genetic characterization of Brachionus clones, and 2) determination of genotypic diversity of cultured rotifers as it may change under different conditions (by controlling biotic and abiotic parameters). Recent advances in molecular genetics have provided the opportunity to apply new, more sensitive techniques to population genetic studies.

Samples of rotifer cultures from hatcheries are separated into clones. These clones are fingerprinted using 2 markers: 1) the mitochondrial 16S/DNA gene and 2) the HSP60 (Heat Shock Protein 60) gene. The HSP60 gene is involved in stress related responses. Mutations in the 16S/DNA gene are considered neutral. This is not necessarily true for the HSP60 gene, where a certain genotype (if displaying a certain phenotype) can be easily selected for in mass cultures of Brachionus, probably depending on the culturing conditions.

Polymorphisms are detected by SSCP technique (Single Strand Conformation Polymorphism) and by DNA sequencing. Within the species Brachionus plicatilis and B. rotundiformis different genotypes (at least 7) have been found using this 16S/DNA marker in a series of Brachionus strains.

Material & Methods

SSCP (Single Strand Conformation Polymorphism)

Description:
DNA from clones cultures is isolated using the method: Harz &#126; Brachionus (16S-DNA specific primers, biotinylated; Brachionus primers) were developed (Sorgeloos et al., 1996). A: 500 bp fragment is amplified, purified, denatured and resolved on polyacrylamide gel (7% T, 20%).

Origin of the clonal cultures

During the Levich (1991) project, several living rotifer populations, from commercial hatcheries, were obtained. From each commercial culture 10 individuals were isolated and cultured to obtain clonal cultures.

Maintenance of clones:
Brevigere females are preserved in a frozen state in liquid nitrogen and the liquid is periodically replaced with fresh frozen liquid. The frozen cultures are maintained at -80°C.

Analytical clones:
Mexico
Philippines
Ecuador
Portugal
Spain (CSIC)
Norway
Kenya
South Africa

Some of the sequenced samples are labelled material. The Mexican, Norwegian and Spanish samples are still present as clonal cultures at the ARC and are used for further research within the project.

DNA Sequencing

Description:
Based on the DNA-sequence data for the 16S-DNA gene, the 16S-DNA gene was sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

15 PCR samples have been sequenced. Their selection was based on the SSCP patterns and fingerprinting of the

Software used to analyze the sequence data:
GeneScan/Geno (Applied Biosystems)
Sequences were aligned (multiple alignment) and a dendrogram was calculated (UPGMA)

Results & Discussion

At the ARC a collection of clones is being built. At present the clones are genetically characterised using the 16S/DNA marker and the SSCP technique. The samples that gave a clearly different pattern on SSCP have been successfully sequenced: 9 different genotypes were found. This mitochondrial 16S/DNA marker is normally used to distinguish species. With the species Brachionus plicatilis and B. rotundiformis different genotypes have been found. These preliminary results confirm the findings of other research groups (Gómez et al., 2002) that are looking at natural populations. Both species should be considered as speciescomplexes. A second marker that will be used in the project is the HSP60 gene. The gene is involved in stress related responses. In the project it will be verified if Brachionus clones carry different HSP60 alleles. In addition it will be verified if these alleles are differentially selected for under certain environmental or culturing conditions. The result so far obtained indicate that the genetic diversity of Brachionus strains in hatcheries is large. It remains to be established whether the local strains are 100% suitable for the purposes they are used for, meaning live feed in aquaculture.

References


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