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

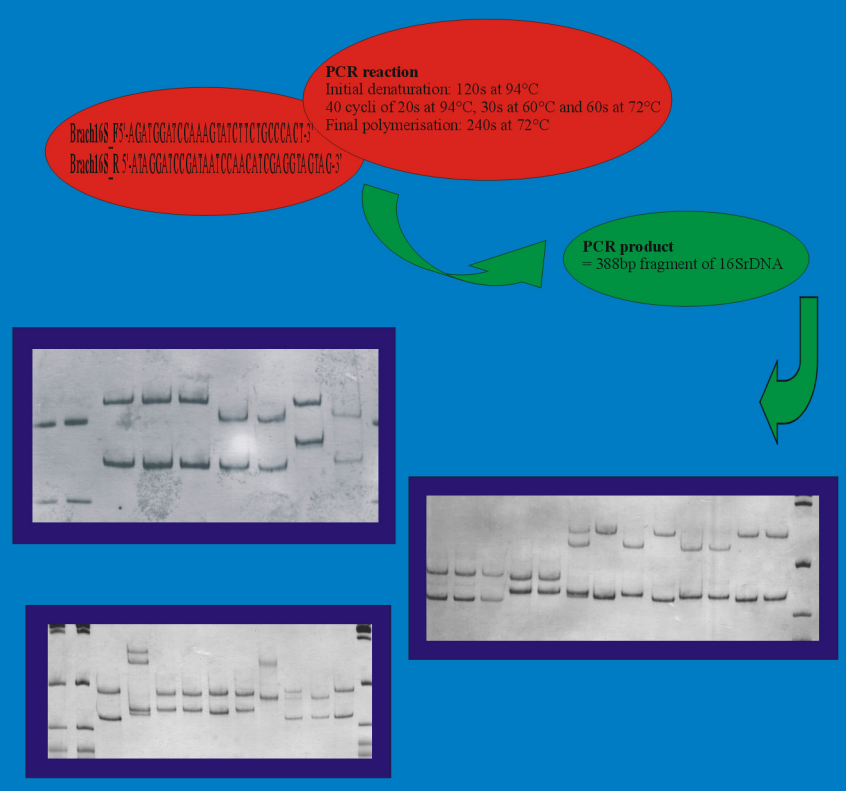
The development of the mass production of high quality fingerlings of marine fish species in Europe was made possible by improvements in the techniques for producing and utilizing live food: rotifers *Brachionus plicatilis* and *Brachionus rotundiformis* (Sorgeloos & Sweetman, 1993; Planas & Cunha, 1999, Dhert *et al.*, 1993; Lavens *et al.*, 1994; Dehasque *et al.*, 1995; Sorgeloos *et al.*, 1995). The rotifer production is still the biggest problem for the fingerling production: the mass culture of these rotifers is very unpredictable (Candreva *et al.*, 1996). Periods with total mortality or reduced reproduction ('crashes') regularly occur. 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 in 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 16SrDNA gene and 2) the HSP60 (Heat Shock Protein 60) gene. The HSP60 gene is involved in stress related responses. Mutations in the 16SrDNA 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 the 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 16SrDNA marker in a series of *Brachionus* strains.

material & methods

SSCP (Single Strand Conformation Polymorphism)

Description

DNA from clonal cultures is isolated wih the CTAB protocol. Brachionus 16S rDNA specific primers (homologous *Brachionus* primers) were developed (Garey *et al.*, 1998). A 388 bp fragment is amplified, purified, denatured and analysed on a poly-acrylamide gel (7.5%, 10°C).



Origin of the clonal cultures

During the Larvi'01 congress in Gent, Belgium, several living rotifer populations, from commercia hatcheries and research institutes, were obtained. From each communal cuture 10 individuals were isolated and cultured to obtain clonal cultures.

Maintenance of clones

Ovigerous females are seperated and placed in falcon tubes (50ml) on a rotor (4 rpm), T=25°C, 25ppt, daily fed with freshly cultured Chlorella. After one week of culture rotifers of each tube are harvested and restocked.

Aalysed clones:

Mexico
Philippines
Ecuador
Portugal
Greece
Spain (CSIC)
Canada
Norway (SINTEF)
South Africa

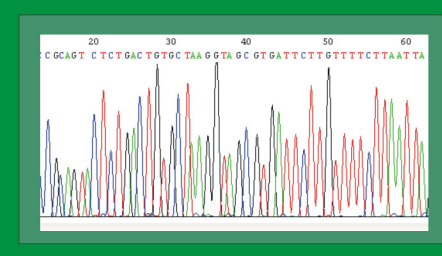
Some of the sequenced samples are lyophilised material. The Mexican, Norwegian and Spanish samples are still present as clonal cultures at the ARC and are used for further research within the project.

DNA Sequencing

Description

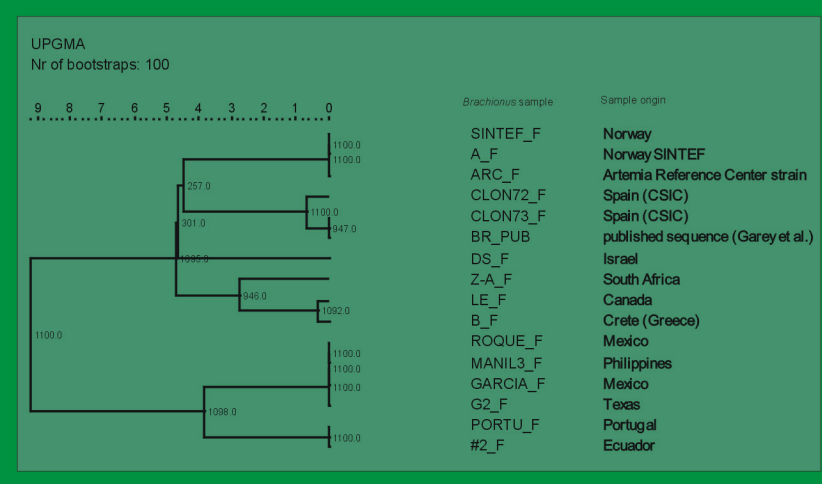
Based on the diversity detected on the SSCP gels PCR fragments of the 16SrDNA gene are sequenced with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

15 PCR samples have been sequenced. Their selection was based on the SSCP patterns: different fingerprints were



Software used to analyse the sequence data = ***Genebase/Kodon*** (Applied Maths)

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 16SrDNA 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 16SrDNA marker is normally used to distinguish species! Within the species *Brachionus plicatlis* and *B.rotundiformis* different genotypes have been found. These preliminary results confirm the findings of other research groups (Gómez et al., 2000, 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 conditionsThe result sofar 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.

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