GENETIC ANALYSIS OF Brachionus plicatilis STRAINS USED FOR AQUACULTURE: PRELIMINARY RESULTS


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INTRODUCTION

Rotifers are considered an invaluable live feed and nowadays rotifer mass cultivation comprises a critical branch of the global marine fish culture industry. Most attention has been focused on Brachionus plicatilis (class Monogononta), a cosmopolitan cyclopid rotifer typically occurring in inland salt lakes and coastal lagoons. A series of studies, initiated in the mid 90s, have given a thorough insight into the taxonomic status and population structures of this commercially important zooplankter [1]. The development of modern molecular tools (microsatellites, DNA sequencing) on adults as well as resting eggs, has unraveled patterns of ecological diversification and adaptation previously undescribed. As a result, Brachionus plicatilis is considered a cryptic species complex comprised of a number of relatively undescribed species.

Increased litter production and culture standardization are highly dependent on determination of mixtures of species with possibly divergent culture requirements and ecological optima. It is therefore obvious that more population genetic approaches are needed which will also greatly benefit applied research on the field. In this regard and within the framework of an EU multi-partner research project we have undertaken the genetic characterization of Brachionus laboratory clones, hatchery samples as well as wild populations through microsatellite genotyping and DNA sequencing. This preliminary survey will be subsequently used for establishing software.

RESULTS - DISCUSSION

Microsatellite genotyping

Laboratory clones

All clones were successfully genotyped at all loci (Table I, Fig. 1). Certain microsatellite loci can be used to distinguish different mixtures of Brachionus clones. For example, locus Bp3 serves as a marker for all possible combinations of clonal mixtures (due to the presence of clone-specific alleles) whereas Bp6b can only discriminate a limited number of mixtures. However, use of microsatellite loci combinations can be used to develop clonal mixtures designed for testing any mixture variability. The same approach could also provide critical information on mixture susceptibility to crashes.

• Koronia Lake population

Genetic variability estimates for the two microsatellite loci analyzed are shown in Table II. Genetic variability levels in the Koronia Lake population are similar to those of other wild populations reported in the literature [1]. However, we report on the presence of two novel alleles, namely 208 and 217, at the Bp3c locus albeit in low frequencies (Table III). Absence of Hardy-Weinberg (H-W) equilibrium (based on a single locus, see Table II) cannot be safely evaluated before more loci on additional alleles are tested.

MicroARTS and METHODS

Microsatellite genotyping

Laboratory clones

Four different clones, namely 10, L1, L3, L7, sent to us from Laboratory of Aquaculture & ARC (Ghent University, Belgium) were investigated. Sample size for each clone was 139, 95, 93 and 34 individuals, respectively. DNA extractions were performed using the Chelex protocol [2] for a battery of 7 microsatellite loci (Bp1b, Bp2, Bp3c, Bp4a, Bp5d, Bp6b and Bp3c) followed conditions described in [3]. Samples were genotyped (Fig. 1) on a LI-COR 4200 DNA Analyzer using the Soyad™ software.

• Wild population

Sixty individuals from Koronia Lake (Thessaloniki, Greece) were also analyzed for 2 microsatellite loci (Bp5d, Bp3c). Results were treated with Genepop and Genetix computer programs.

Sequencing the COI mitochondrial region

A total of 14 clones, originating from 6 laboratories (Mexico, Belgium, Japan, SpainA, SpainB, Vietnam) and 2 hatcheries (Norway, Italy), all raised in ARC were investigated. Sample size for each clone was 139, 95, 93 and 34 individuals, respectively. DNA extractions were performed using the Chelex protocol [2]. PCR amplification for a battery of 7 microsatellite loci (Bp1b, Bp2, Bp3, Bp4a, Bp5d, Bp6b and Bp3c) followed conditions described in [3]. Samples were genotyped (Fig. 1) on a LI-COR 4200 DNA Analyzer using the Soyad™ software.

TABLE I. Genotyping results of clones 10, L1, L3 & L7

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele Frequency</th>
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<tbody>
<tr>
<td>Bp5d</td>
<td>276/300 (0.920)</td>
</tr>
<tr>
<td>Bp3</td>
<td>199/200 (0.995)</td>
</tr>
<tr>
<td>Bp3c</td>
<td>199/210 (0.935)</td>
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</tbody>
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REFERENCES