Application of a Multiplex PCR with Specific PCR Primers for the Detection of the Genus *Paramecium* and the *Paramecium aurelia* Complex

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Summary. The representatives of the genus *Paramecium* are well-studied ciliates and can be used in water quality assessment and the determinations of saprobic levels. For these applications, a clear and unambiguous identification of ciliate assemblages is essential, which is typically based on morphological characters requiring a sound taxonomic knowledge and experience in species determination including microscopic identification of both living and stained specimens. Therefore, we developed and applied specific PCR primers for the detection of species belonging to the genus *Paramecium* and the *Paramecium aurelia* complex. These primers were successfully tested with different *Paramecium* species including representatives of the *P. aurelia* complex as well as closely related species like *Frontonia* sp. and *Tetrahymena* sp. in both experimental and environmental samples. These primers can be used in a simultaneous approach achieving fast and reliable results with regard to determination of ciliate community and water assessment.

Key words: Multiplex PCR, *Paramecium*, saprobic level, species specific primers.

INTRODUCTION

To date, 17 morphospecies have been described in *Paramecium* (Fokin et al. 2004). Several of these species have been used in water quality assessment and determination of the saprobic level (Berger et al. 1997). For example, the presence of species of the *P. aurelia* complex indicate good quality of effluent to slightly polluted waters (Curds and Cockburn 1970). By contrast, the presence of *P. putrinum* is an indicator of heavily polluted and oxygen-deficient water (Berger et al. 1997).

To draw conclusions about water quality using the presence or absence of the specific *Paramecium* species, clear and unambiguous determinations are required. These determinations have traditionally been carried out by the evaluation of morphological characters in combination with different silver-staining methods. However, these exact identifications require a profound morphological knowledge (Fokin et al. 2004). Investigations of ciliate communities were soon ex-
panded by the use of molecular markers after their taxonomic significance had been realized. One approach is based on the analyses of clone libraries followed by sequence comparisons with GenBank data (e.g. Epstein and López-García 2008, and references therein). This sequencing approach is time consuming and expensive.

An alternative approach to identification is the use of diagnostic species- or clade-specific primers. Such diagnostic primers have been used especially of pathogenic protists; e.g. for the detection of Legionellae diagnostic primers have been used especially of pathogenic species- or clade-specific primers. Such a sequencing approach is time consuming and expensive. And López-García 2008, and references therein). This sequence comparisons with GenBank data (e.g. Epstein is based on the analyses of clone libraries followed by

MATERIAL AND METHODS

Origin of samples
Genomic DNA of all Paramecium isolates was kindly provided by D. Barth (Leipzig), C. Zschornack (Dresden), and E. Przyboś (Kraków, Poland).

Development of PCR-primers for the Multiplex-PCR

Based on an alignment using ClustalX (Thompson et al. 1997) of all available 18S rRNA gene sequences of Paramecium spp. (13 species) and 35 further ciliate species out of all ciliate classes, a genus-specific reverse primer (5’-GGCTGATGACCTRTGCCTTACA-3’) was developed. This primer targeting Paramecium was checked for cross matching with other DNA sequences using the program BLAST (Altschul et al. 1990), and then paired with a universal eukaryote specific SSU rDNA forward primer (Elwood et al. 1985) to amplify a 1564bp fragment. Additionally, for each investigated isolate a second amplification with two universal SSU rDNA primers (Elwood et al. 1985) was performed spanning the complete SSU rDNA. The PCR was done with 0.2 µM of each primer (18SF and Paramecium R), 1.5 U Taq polymerase (Fermentas), 10 × PCR buffer supplied with Taq, 200 µM dNTPs and 3–5 µl genomic DNA in a total volume of 50 µl. The reactions were carried out in a Mastercycler (Eppendorf) using the following PCR program: 5 min. denaturation at 95°C, followed by 35 cycles (45 s denaturation at 94°C, 1 min. annealing at 58°C, 1 min. elongation at 72°C), and a final elongation step at 72°C for 10 min. Amplifications were checked by gel electrophoreses.

RESULTS AND DISCUSSION

The detection of the genus Paramecium as well as the Paramecium aurelia complex was carried out by applications with newly developed specific primers in a Multiplex PCR. First, the Paramecium-specific primer was tested in combination with a universal 18S forward primer. In total, 16 different species were used for this analysis and each investigated species was tested twice. A first amplification was done as a positive control with two universal SSU rDNA primers that amplified the whole 18S rDNA gene, whereas a second amplification was performed with the new Paramecium-specific primer. The results of these amplifications and the following gel electrophoreses are shown in Figs 1A, B.

The lanes labeled with even numbers show the amplifications with the Paramecium-specific primer, and the odd numbers the positive controls (whole SSU rDNA).
Detection of *Paramecium* by Multiplex PCR

All tested *Paramecium* species (lanes 1 to 22) showed clear bands in both amplifications. Furthermore, the lengths of the PCR products corresponded to the expected size of 1564bp with the *Paramecium*-specific primer, and 1800bp for the whole SSU rRNA. In addition to the *Paramecium* isolates, six related species of other ciliated genera were investigated. These species showed bands (lane 23 to 34) only after amplification with the universal SSU rDNA primers, but no visible bands with the *Paramecium*-specific primer. Only members of the genus *Paramecium*, therefore, yielded bands. All other tested species including related species generated no amplification products and we hypothesize that also other non-tested ciliate species will be discriminated.

After the application of the *Paramecium*-specific primer, a Multiplex PCR was performed using the *P. aurelia* primer (Fig. 1C).

The first five lanes show the results of representatives of the genus *Paramecium* that do not belong to the *P. aurelia* complex. As expected only the 1500bp long *Paramecium*-specific fragment from the first round PCR was visible. By contrast, amplifications using DNA from members of the *P. aurelia* complex (lane 42 to 47) clearly showed both bands; i.e. the *Paramecium* - and the *P. aurelia*-specific PCR-products. All non-*Paramecium* species as well as the negative control yielded no products. A Multiplex PCR using bulk genomic DNA isolated from a constructed wetland produced the same two bands (data not shown). This
result is in agreement with light microscopy control of the wastewater sample in which we identified members of the *Paramecium aurelia* complex (between 2 to 10 cells per ml).

In summary, the established Multiplex PCR – using *Paramecium* - and the *P. aurelia*-specific primers – produced the expected results for the tested isolates. Furthermore, the comparison with SSU rRNA data from all species out of the *Paramecium aurelia* complex (Catania et al. 2009) revealed no sequence differences within the primer binding site. This suggests optimal results also for other *P. aurelia* species that we did not test. Furthermore, the application of the developed primer set on related species like *Frontonia* sp. or *Tetrahymena thermophila* was discriminative. Based on this result, we assume that distantly related species will also yield no products using this primer set.

The development of these tools represents an easy, reliable, and cheap method for the determination of species in the genus *Paramecium* and the *P. aurelia* complex. In combination with already available species-specific primers fast insights into ciliate assemblages can be gained, especially by non-morphologists. For more detailed analyses of the different *Paramecium* species within an environmental sample, for each species specific primers have to be developed in order to increase the spectrum of diagnostics devices for the assessment of water quality and saprobic levels.

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**REFERENCES**


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