DETECTION OF MICROALGAL RESTING CYSTS IN EUROPEAN COASTAL SEDIMENTS USING A PCR-BASED ASSAY

IDENTIFICAZIONE DI CISTI DI RESISTENZA DI MICROALGHE IN SEDIMENTI DI AREE COSTIERE EUROPEE TRAMITE LA TECNICA MOLECOLARE DI PCR

Abstract - A PCR-based assay was developed and applied to sediment samples for the detection of different cysts belonging to dinoflagellates and raphidophytes in European coastal areas. Higher values of positive detection for target cysts were obtained by PCR than with microscopy. This molecular methodology permitted a fast and specific detection of target cysts in sediment samples.

Key-words: HAB species, Mediterranean Sea, ribosomal genes, resting stages, sediments.

Introduction - Studies on resting cyst diversity and distribution have been hampered by difficulties in the identification of species-specific resting stages, since dinoflagellate cysts do not always have species-specific morphological features. PCR techniques can be applied to samples from sediment cores containing a variety of cysts. Such molecular-based assays can accurately and rapidly identify a variety of specific taxa in the sediments, overcoming the problem of taxonomic identification by microscopy (Godhe et al., 2002; Bowers et al., 2006; Kamikawa et al., 2007). In this study, a PCR-based assay was developed and applied to sediment samples collected from several European coastal areas for the detection of resting cysts from several taxa. The specificity and sensitivity of each assay was determined and comparisons of the qualitative determinations of the PCR analysis and optical microscopy were made.

Materials and methods - Surface sediment samples were collected during the years 2006 and 2007 from 38 sampling stations located at 11 different sites in the Mediterranean and Baltic Sea and the East Atlantic. Cyst sample collection, purification and microscopy morphotype identification are described in Penna et al. (2009). Genomic DNA extraction of sediment samples, and PCR amplification detection assay as well as the specificity and sensitivity of the assays were accomplished as described in Penna et al. (2007, 2009).

Results - PCR amplifications using primers designed in the highly variable ITS regions and conserved 5.8S and LSU (D1/D2) ribosomal genes of different dinoflagellate genus and species gave amplified fragments of different base pair lengths. The specificity of the genus- and species-specific primers was assessed both by multiple alignment on the silico platform of BLAST and by PCR amplification of the genomic DNA from each genus and species in the presence of mixed non-
target taxa clonal strain DNA together with target species clonal strains. The target genus- and species-specific primers showed high specificity in all selected PCR-based assays.

The sensitivity and the absence of inhibitors of the PCR-based assay using specific primers were assessed on the plasmid target cloned sequence of a murine retroviral complex.

The PCR assay on plasmid DNA was sensitive enough to detect ten copies of the cloned sequence in the presence of 1 ng of sediment DNA as background. The sensitivity of the PCR assay was also assessed using genomic DNA as a template. The sensitivity of the PCR-based assay carried out on genomic DNA corresponded to the specific PCR amplification of 1 pg. PCR amplification was performed on several Lugol-fixed sediment and sediment trap samples collected during the years 2006 and 2007 along the coastal areas of the Mediterranean Sea, north-western coast of Spain and Baltic Sea to detect the presence of either dinoflagellate resting cysts belonging to the genera *Alexandrium* and *Scrippsiella* and species *A. minutum*, *A. tamarense*, *A. catenella*, *A. taylori*, *Gymnodinium catenatum*, *G. nolleri*, *Lingulodinium polyedrum*, *Protoceratium reticulatum* and *S. hangoei* or raphidophyte resting cysts belonging to the species *Fibrocapsa japonica*. These samples contained mixed cyst communities including the target morphotypes, with total cyst abundances ranging from $3.0 \times 10^4$ to $7.9 \times 10^5$.

The amount of target taxa in the analysed samples ranged from $1.0 \times 10^1$ to $2.3 \times 10^5$ of cysts. Amplification reactions were performed on sediment samples using genus-
and species-specific primers. Nested PCR amplification reactions with eukaryotic
specific primers targeting the 5.8S-ITS and LSU rDNA regions first, and then
amplifying the genus- or species-specific regions, always gave positive PCR products
of the expected size for each genus and species from the genomic DNA in sediment
samples.

The PCR assay detected the presence of the resting stages belonging to different
dinoflagellate taxa even if target cysts were not observed in the sediment and
decidum trap samples by microscopy examinations.

The PCR assays were positive for the presence of cysts of the genera *Alexandrium*
and *Scrippsiella* and the species *A. minutum*, *A. tamarense*, *G. catenatum*, *G. nolleri*,
*L. polyedrum*, *P. reticulatum*, and *S. hangoi*.

The resting cysts of species *A. catenella* and *A. taylori* were never detected in the
sediment samples examined by PCR assay and microscopy. Considering the total
number of sediment and sediment trap samples processed per each area, the results
of the positive detection of the *Alexandrium* and *Scrippsiella* and *A. minutum*, *A.
tamarense*, *L. polyedrum*, *P. reticulatum*, *G. catenatum*, *G. nolleri*, *S. hangoi* and
*F. japonica* were compared based on the two methods used, PCR technique and
microscopy (Fig. 1).

The positive PCR amplifications and microscopy identifications were equal for
*F. japonica* cysts; in contrast, the species specific identification of *A. tamarense*,
*G. catenatum* and *G. nolleri* was confirmed by the molecular method only. The
positive detection values obtained by PCR assay were higher than microscopy
determinations by 48% for *A. minutum*, 36% for *P. reticulatum*, 15% for *Alexandrium*

![Graph](image)

**Fig. 2** - Number of samples that were positive by both methods (PCR-based assay and microscopy)
for the detection of different dinoflagellate and raphidophyte cysts (■) and vegetative cells
(●) in sediments and seawater samples, respectively. The diagonal line indicates equal
detection of both methods in sediments and seawater samples. Sediment samples were
collected in European coastal waters during 2006 and 2007. Positive PCR and microscopy
determinations on seawater samples were derived from Penna et al. (2007).
spp. and *Scrippsiella* spp. and 10% for *L. polyedrum* cysts. Furthermore, when PCR and microscopy methods were applied to sediments and seawater for the detection of target taxon as resting or vegetative stages, the PCR method produced a higher number of positive detections compared to the positive microscopy determinations both in sediment and seawater substrates (Fig. 2).

**Conclusions** - In this study, the specificity and sensitivity of the PCR-based technique for the detection of target cysts in marine sediments were established. The PCR method permitted higher detection efficiency than the microscopy method, illustrated by the higher positive percentage identification of the harmful target cysts in sediments.

Knowledge of species composition is important to understand bloom events in the coastal areas.

In the future, the PCR method could be used for mapping the distribution of target species cysts in coastal sediments, particularly given its high specificity and sensitivity.

**References**


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