MOLECULAR ANALYSIS OF *GYRODINIUM INSTRIATUM* EXCYSTMENT CELL USING FTA TECHNOLOGY

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Abstract
Gyrodinium sp. (BgT3C2) strain isolated from a resting cyst in sediment from Bougrara lagoon (Tunisia) was used to investigate the utility of FTA Technology for the protection and purification of nucleic acid. We have used FTA cards to obtain the sequence of the LSU rDNA D1-D2 region from very low concentrated BgT3C2 strain culture. Nucleotide sequence analysis supported and confirmed identification of BgT3C2 strain to *Gyrodinium instriatum.*
Keywords : Biotechnologies, Genetics, Phytoplankton.

Introduction
Using molecular approaches revolutionised the identification and taxonomic of the organisms with their genetic characterization, mostly based on nuclear ribosomal DNA markers [1]. DNA-based studies usually require highly concentrated clonally cultures. However, the culturing process is not always suitable. FTA enables the collection, storage and purification of nucleic acids from a wide variety of biological sources. The aim of this study is to investigate the suitability of FTA technology to obtain specific genes sequences for molecular identification of marine dinoflagellates avoiding highly concentrated cultures. Molecular data were compared to morphological characters of BgT3C2 strain to study the reliability of FTA in the species identification.

Materials and methods
Gyrodinium sp. cyst was isolated from sediment by core sampler from Bougrara Lagoon (Tunisia). The culture was maintained in L1 medium on a multiwell plate and grown at 22°C (12:12 L:D, 50 μEm^{-2}s^{-1}). A monoclonal culture of *Gyrodinoium* (BgT3C2) was subsequently established and the growth phase of the BgT3C2 strain determined every 1-2 days.

Genomic DNA was extracted using FTA technology (Transgenomic) according to the manufacturer's recommendations. 65 μl of the BgT3C2 strain culture was applied to FTA cards. After six months of storage, punches (2 mm) were removed from the FTA cards, washed and used as PCR template for the amplification of the LSU rDNA D1-D2 domains. The amplified rDNA fragment was purified and sequenced. PCR and sequencing reactions were performed using D1R, D2R, D1C and D2C primers [2].

Results and discussion
Two different motile cell types emerged after 11 dormancy days from cysts in artificial medium L_1. The morphology of the cyst and motile cell of BgT3C2 strain seems to be similar to those described for *Gyrodinium instriatum* [3]. The lag phase of *Gyrodinium instriatum* lasted only for one day. The exponential stage lasted 30 days and maximum abundance was 40cells/ml. This low abundance prevent the use standard molecular method to identify the strain.
Using FTA cards, the D1-D2 region of rDNA LSU was successfully amplified from low density cultures of BgT3C2 strain. The D1R/D2C PCR product was purified and sequenced with D1R, D1C, D2R and D2C primers. We obtained a new sequence of approximately 650 bp using FTA cards as the source of PCR template. The new sequence of the LSU rDNA D1-D2 region of BgT3C2 strain has been deposited in GenBank under the accession number DQ997780. The LSU rDNA sequence analysis confirmed that the identity of the *Gyrodinium instriatum* cysts, BgT3C2 strain.

Conclusion
Using FTA cards, allow obtaining specific gene sequences from marine dinoflagellates even at low abundance.

References
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