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Conference Abstract

Characterization of marine eukaryotic biofilms at offshore wind farm sites: assessment of DNA extraction methods and marker gene used for metabarcoding approaches

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Abstract

Among marine lifestyles, biofilms are considered as diversified communities embedded in complex exopolymers whose development depends on several factors, related to both environmental conditions and physical-chemical characteristics of substrates (Antunes et al. 2019, Bellou et al. 2012). For the maritime industry, bio-colonization and its impact on human activities were well-described (Schultz et al. 2011). However, this phenomenon represents a new challenge in Renewable Marine Energies (RME) due to their specificities (materials, structures, localization…). In particular, macro-organism assemblages appeared to include a wide variety of eukaryotic groups but the literature is sparse considering the sequencing of eukaryotic diversity in comparison to those of bacterial communities (Briand et al. 2018, Dang and Lovell 2000, Salta et al. 2013). As a matter of fact, the very small size of some of the eukaryotes and/or their insufficient morphological discernible features appear to considerably limit their detection and identification, leading to underestimate their diversity (Carugati et al. 2015). When talking about molecular approaches, analysis of eukaryotes also represents a challenge because such organisms possess resilient cellular structures which can give poor DNA extraction yield (Hermans et

al., 2018Hermans et al. 2018). In addition, SSU rRNA in eukaryotes fails to be as universal as for prokaryotes (Bik et al. 2012, Medinger et al. 2010). However, the use of marker genes from environmental DNA, when focused on the targeted eukaryotic community, remains critical to decoding the complexity of marine biofilms diversity.

In this study, four extraction methods, including a preliminary mechanic cell lysis, both soil and biofilm kits, and global approaches, have been compared. We also examined the coverage and the identification capability of several primers to characterize eukaryotic communities colonizing three plastic surface types (polyvinyl chloride, HD polyethylene, and polyamide) which have been immersed in several locations along the French Mediterranean and Atlantic coasts. Sequence quality and number remain the same whatever the extraction method. However, the richness and community structure were clearly affected regardless of the sample type (Figure 1). Finally, two kits (PowerMaxSoil, and PowerBiofilm kits) evaluated in this study were considered as the most powerful overall.

Secondly, we amplified and sequenced short fragments of two genes: one region of the mitochondrial Cytochrome Oxidase subunit I (COI) and five variable regions of the 18S small subunit ribosomal DNA (rDNA) gene (V1V2, V4TAR, V4UNI, V7, and V9). The Chao1 index was considerably lower for the CO1 gene compared to those of the 18S rDNA regions. The V4TAR and V7 regions showed a significant highest richness, followed closely by the V1V2 and V9 regions. The 18S rDNA gene sequences were dominated by microeukaryotes whereas the COI sequences were dominated by macro-organisms. Each of the 18rDNA primer pairs also exhibited dissimilar community structures although the dominant taxa seemed to be common.

To conclude, our results provided a global assessment of tools dedicated to the description of the diversity of marine eukaryotes biofilms from three surfaces used in the design of RME. Among the four extraction methods described here, PowerMaxSoil and PowerBiofilm kits allowed recovering the highest diversity. COI and 18S rDNA gene sequencing covered different groups including at high taxonomic levels. Despite limitations, metabarcoding will help in the characterization of marine biofilms diversity on RME. Especially, it may be relevant to use primers targeting these two genes to better cover the eukaryotic diversity.

Keywords

Metabarcoding, marine biofilms, *Cytochrome c oxidase I*, Eukaryotic communities, 18S ribosomal RNA gene

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