Extended abstract

Brilliant Marine Research Idea 2021

1. General information

Title of the idea	Integration of taxonomic and phenotypic fingerprints of marine plastic degrading communities
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2. Brilliant Marine Research Idea

Extended abstract

The successful results (thanks to the BMRI grant)

Flow cytometry is a powerful tool to monitor microbial communities as it allows to follow changes in the phenotype of the community in combination with absolute abundances in high throughput at a very low price. This information can be combined in a so-called phenotypic fingerprint that can be used for diversity-analysis[1-3].

In this research we used the phenotypic fingerprint to monitor how microbial communities change over time when exposed to plastics. More specifically, a trickling filter was set up in which an enriched seawater culture, obtained in previous enrichments, was trickled over plastic material. Four reactors were set up, with two different types of plastics, in duplicate. The inoculum originated from the enriched seawater community on the corresponding plastics. Effluent samples for flow cytometry analysis were taken every 2 days, with exception of weekends. After sampling, samples were subdivided for either direct flow cytometry measurements or fixation. Staining was performed with either 1 vol% SYBR® Green I (SG, 100x concentrate in 0.22 µm-filtered DMSO, Invitrogen) or with 1 vol% 4′,6-diamidino-2-phenylindole (DAPI, 3 mM concentrate in 0.22 µm-filtered DMSO, Invitrogen). Samples were also taken for 16S rRNA gene sequencing, but less frequently.

As phenotypic fingerprinting is not always straightforward to link with taxonomic diversity, we also combined flow cytometry (FCM) with Fluorescent In Situ Hybridization (FISH). This allowed us to make a combination of taxonomic (FISH) and phenotypic (FCM) information to obtain an integrated fingerprint. FISH probes were chosen based on literature. Taxonomic groups that were reported to have importance in plastic biodegradation or were abundant in the plastisphere, in this case Alpha-Proteobacteria and Gamma-Proteobacteria, were selected [4,5]. As a control, probes targetting all bacteria were used.

On these 4 datasets (fresh samples stained with SG, fixed samples stained with SG, fixed samples stained with DAPI and fixed FISH samples counterstained with DAPI), diversity analysis was performed using statistical packages like *vegan* (v2.5.6)[6] and *Phenoflow* (v1.1.2)[7]. Fingerprinting was performed

with the *phenoGMM* function integrated in *Phenoflow* based on Rubbens et al. (2020)[8]. From this model, the beta-diversity, the diversity between samples, based on the Bray-Curtis dissimilarity matrix could be calculated. The results showed that the beta-diversity couldn't discriminate the two reactor types (thus plastic types) for the fresh samples nor for the fixed samples stained with SG or DAPI. However, there was a clear difference between the inoculum samples and the later timepoints for the fresh samples. The fixed FISH samples on the other hand, showed a clear distinction between the two reactor types, but no clear distinction between inocula and later timepoints. This was confirmed by the sequencing data, which also indicated that the most important difference between samples was coming from the reactor, and thus plastic types.

Our results confirmed the hypothesis that the combination of taxonomic (FISH) and phenotypic (FCM) information improves the microbial fingerprint. By labelling specific taxonomic groups, we can not only add an extra dimension to the fingerprint, but we can also see which groups of organisms are enriched and might play a role plastic biodegradation. This methodology could be a valuable way to answer research questions concerning microbial community dynamics.

The stumbling blocks on the way

Before we got to the results we could present above, a lot of optimization and failed experiments preceded. Firstly, we changed our very labor-intensive and time-consuming workflow to a more automated one, which needed little optimization but turned out to be a great upgrade. Secondly, we performed some optimization for hybridization conditions for our probes, staining protocols for counterstaining and selecting the right fluorescent markers for our probes. Thirdly, we tried the protocol on several samples sets, but often problems of low cell counts made it impossible to use the results for downstream data analysis.

Originally, it was planned to sort out and sequence the samples to validate the specificity of the probes. As we already had the 16S rRNA sequencing data of the samples, it was decided not to sort out the samples, but instead compare the abundances found with FISH with the abundances found in the sequencing data. This was a more cost- and time-efficient choice.

Isolation with FISH-sorted cells was not performed. Live-FISH has been described successfully in literature, but the success rate was very low and only very low percentages of bacteria that survived the whole protocol[9]. This was also confirmed by our initial tests. We first tried to identify the most detrimental step in the protocol, and tried to make it less severe. At the same time, we were working on the automated upgrade, which could also have several advantages for the live-FISH protocol. In the end we were not able to try this out yet, because we gave the priority to the main goal of the research: a combination of taxonomic (FISH) and phenotypic (FCM) information to obtain an integrated fingerprint that can be used for improved diversity analysis.

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