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SEAFRONT

Synergistic Fouling Control Technologies

Deliverable 5.52: Molecular genetics sequencing of slime collected from up to eight immersion sites and up to six coatings.

Delivery date: M48 (January 1st 2018)

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1 Introduction

Deliverable 5.52 has been delivered with the aim being to undertake molecular genetics sequencing of marine biofilms collected from up to eight immersion sites and up to six coatings.

2 Partners involved

International Paint Marine & Protective Coatings (IP)
University of Bristol (BRIS)

3 Description of technology delivered

Related experimental results on static immersion boards and laboratory-based flumes were reported in WP4 and deliverables therein. Here we report on in-situ performance testing of nine commercial coatings in five locations around the UK and waters of the North Sea. The purpose of the experiments was to assess the effect of the coating type (factor) on the microbial composition of the biofilms forming on commercial vessels and structures. Sampling details are shown in Table 1.

Table 1. Details of vessels and structures used for in situ performance testing

Vessel	Port boards	Starboard boards	Type	Operational Area	Trial start-date	Sampling date
Wansbeck	3	3	Fishing vessel[1]	North Sea, Port of Blyth	21/09/15	11/10/16
Halton	3	3	Dive vessel[1]	North Scotland - Norway	19/01/15	10/01/17
Donald Searle		3	Sailing[1]	English Channel	24/02/15	08/02/17
Discovery	3		Sailing[1]	English Channel, UK wide + freshwater	24/11/14	11/11/16
Raft	N/A		Structure[2]	Chichester Marina, UK	04/03/15	19/07/16, 10/11/16

[1] In situ performance testing for D5.27, [2] Coated device testing, D5.46

4 Methods

Four deep sea marine biocidal coatings – referred to as BAF1, BAF2, BAF3 and BAF4, three biocidal coatings specifically designed for yachts and pleasure craft – referred to as BAF-Y1, BAF-Y2 and BAF-Y3 and two fouling release coatings – referred to as FR 1 and FR2 were applied to vessels and boards (Figure 1). Vessels had up to six replicate test patches applied (see Table 1).

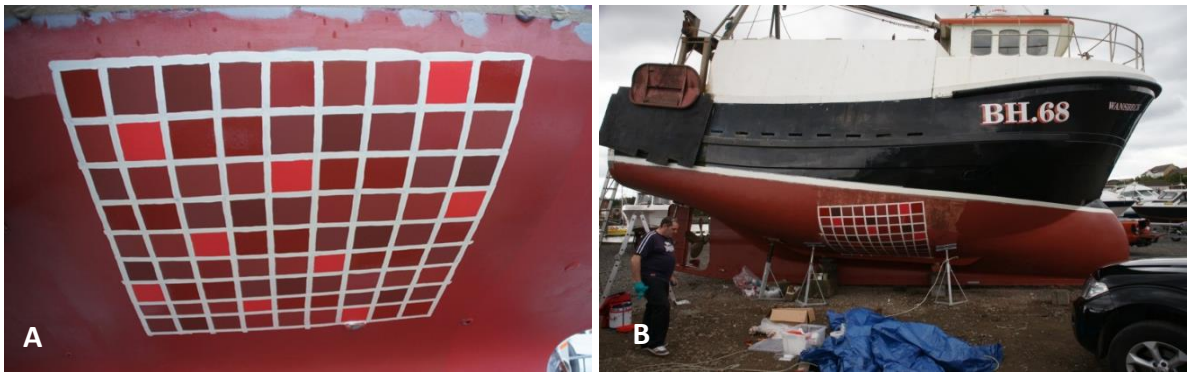

Figure 1. Experimental design used in the study. A – Latin square board with the nine coatings. B – Test area located on the starboard hull of a vessel.

Slime samples were scraped from test patches and DNA extracted using Power Biofilm kits (Qiagen) as described previously. The raft at Chichester Marina was used as a representative static structure/device as those from the associated SMEs and end users were not immersed for sufficiently long periods to result in significant biofilm formation. Bacterial biofilm composition was assessed by amplifying the V3 region of the 16S Ribosomal RNA coding genes. Amplicons were barcoded and sequenced on an Illumina MiSEQ. Taxonomic analysis was performed using QIIME 2, with a Naive-Bayes classifier trained on 16S reference reads extracted from the Greengenes 97% Operation Taxonomic Unit (OTU) dataset. Features that had a frequency lower than 10 across all samples, and samples with less than 1000 features were filtered. In addition, the chloroplast and mitochondria rRNA observed in the data that was also removed prior the analysis. Coating-specific differences were tested for statistical significance with Bonferroni-corrected Kruskal-Wallis pairwise tests and ANOSIM in SPSS v24 and QIIME2 respectively.

5 Results and Discusion

We found no significant difference taxonomic diversity of biofilms (slime) across the three broad coating categories (BAF, FR and BAF-Y) – Figure 2. We found instead that the vessel/structure location was the key driver for observed difference in microbial community (Figure 3).

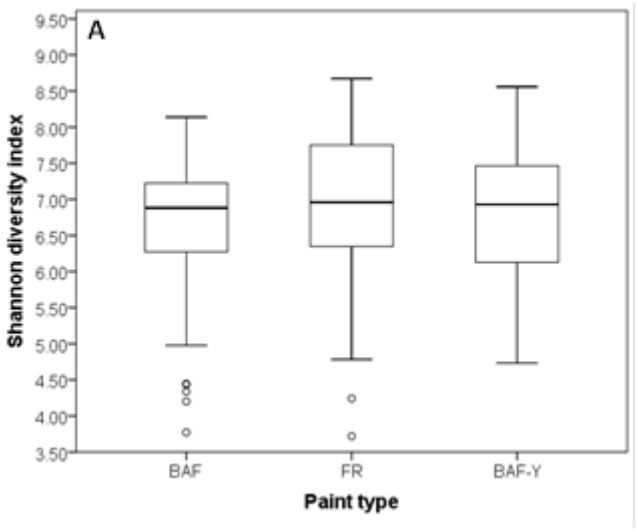

Figure 2. Boxplot of Shannon taxonomic diversity measures for the three broad coating categories. There was no difference between commercial biocidal coatings (BAF), fouling release coatings (FR) and yacht-specific biocidal coating (BAF-Y) in terms of their effect on fouling-community diversity.

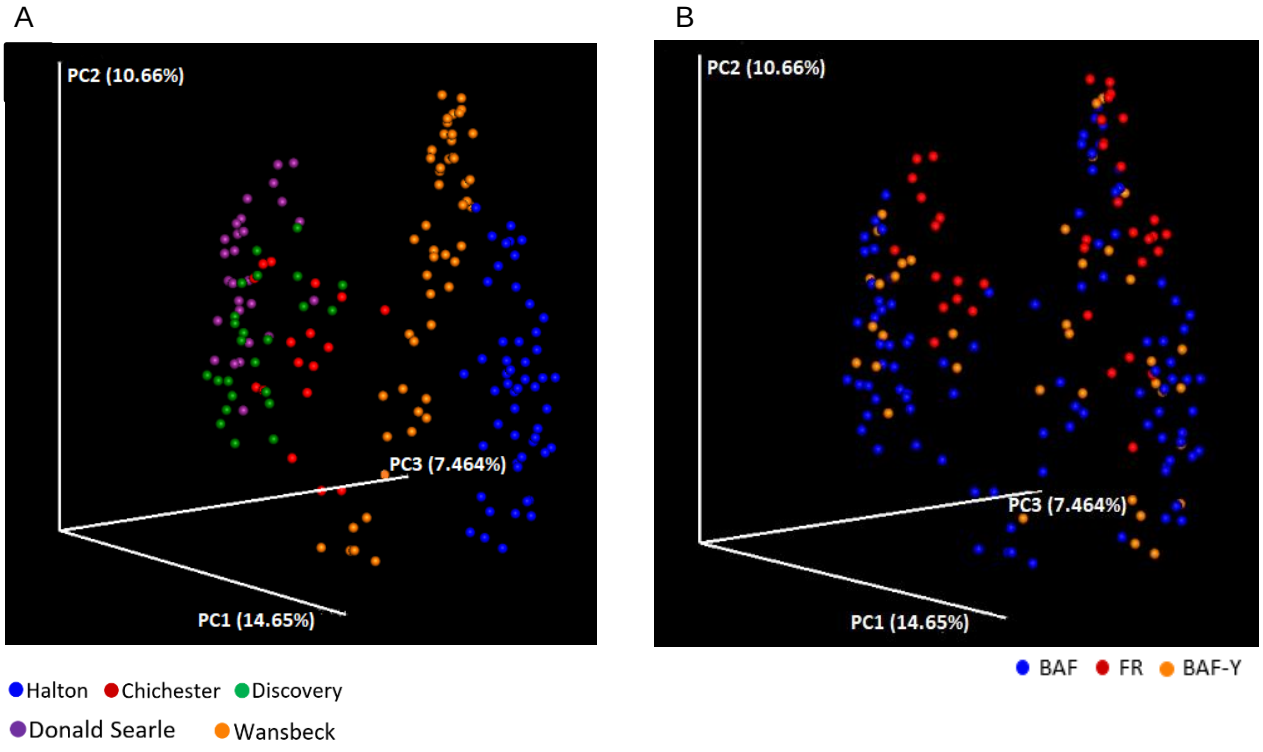

Figure 3. PCoA plot of samples, colour-coded by location. The plots shows that vessel location (A) is well correlated with PC1 (15% of sample variance) whereas coating type (B) did not correlate with observed variance. Locations: Discovery and Donald Searle & Chichester (Raft) English Channel (southern UK), Wansbeck = Northern England, Halton = Scotland/Norway.

The PCoA representation shows us the similarity of the microbial composition of each sample, with each dot representing a single sample from a particular vessel and coating type, and dots close to one another representing samples with similar microbial communities.

The results from single static immersion sites and laboratory flume experiments (reported in WP4) revealed strong coating-specific effects on microbial diversity. In contrast, these results from service vessels operating across a wide range indicate that geographic location is a key driver for microbial community composition in marine biofilms.

6 Public Deliverables

The sequence data produced have been uploaded to the MG-RAST public repository and will be released upon acceptance of the associated manuscript (in preparation). Flat test panels have been prepared with the three down selected prototype candidates to test

7 Conclusions

Molecular genetics sequencing of biofilm collected from five immersion sites/devices/structures from nine coatings has been completed showing that there was no significant difference in taxonomic diversity of biofilms (slime) across the three broad coating categories (BAF, FR and BAF-Y) but instead vessel/structure location was the key driver for observed difference in microbial community.

8 References

None