Long-term microfouling on commercial biocidal fouling control coatings

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The current study investigated the microbial community composition of the biofilms that developed on 11 commercial biocidal coatings, including examples of the three main historic types, namely self-polishing copolymer (SPC), self-polishing hybrid (SPH) and controlled depletion polymer (CDP), after immersion in the sea for one year. The total wet weight of the biofilm and the total bacterial density were significantly influenced by all coatings. Pyrosequencing of 16S rRNA genes revealed distinct bacterial community structures on the different types of coatings. Flavobacteria accounted for the dissimilarity between communities developed on the control and SPC (16%) and the control and SPH coatings (17%), while Alphaproteobacteria contributed to 14% of the dissimilarity between the control and CDP coatings. The lowest number of operational taxonomic units was found on Intersmooth 100, while the lowest biomass and density of bacteria was detected on other SPC coatings. The experiments demonstrated that the nature and quantity of biofilm present differed from coating to coating with clear differences between copper-free and copper-based biocidal coatings.

Keywords: bacteria; diatoms; microfouling; biocidal coatings; diversity; pyrosequencing

Introduction

Biocidal coatings are widely used on ships, boats and other marine structures to control biofouling (Yebra et al. 2004). Following the phase out of tributyltin (TBT) products, cuprous oxide is used as the main biocide in most commercially available biocidal coatings, most often in conjunction with co-biocides such as zinc pyrithione, copper pyrithione and/or zineb. These are selected due to their positive environmental profile, cost effectiveness, efficiency and endurance (Yebra et al. 2004; Finnie 2006). Additionally, ‘copper-free’ coatings are becoming increasingly available, using organic biocides such as tralopyril or pyridine triphenylborane, again usually in conjunction with the aforementioned co-biocides. Biocidal coatings can generally be grouped into three major types: controlled depletion polymer (CDP), self-polishing hybrid (SPH) and self-polishing copolymer (SPC) coatings (Finnie & Williams 2010). The ability of the coating to control fouling is influenced by the chemistry of the paint binder and the type of biocide present in the coating. These in turn determine the polishing behaviour of the coating and its ability to control the biocide leaching rate (Yebra et al. 2006a, 2006b; Finnie & Williams 2010; Bressy et al. 2010).

The unique physicochemical properties of biocidal coatings affect the formation of microbial biofilms (Whitehead & Verran 2009; Van Mooy et al. 2014). The development of such biofilms in turn depends on the types of microbes present and environmental conditions (Yebra et al. 2006b). Different microbes also have different dimensions and differential growth rates, which affect the drag and shear stress of the biofilmed biocidal coatings (Howell 2009). Microbial fouling communities consist mainly of numerous species of bacteria and diatoms that can positively and/or negatively interact with each other (Railkin 2003; Dobretsov 2010; Salta et al. 2013; Mieszkin et al. 2013) and significantly enhance or inhibit the settlement of invertebrate larvae and algal spores (Mitchell & Maki 1988; Maki 2002; Huang & Hadfield 2003; Qian et al. 2007; Hadfield 2011).

Although the study of biofouling on biocidal coatings has a long history (WHOI 1952; Callow 1986; Yebra et al. 2006b; Chen et al. 2013), very few studies have assessed the composition of marine biofilms on different types of biocidal coatings (Dempsey 1981; Cassé and Swain 2006; Molino, Campbell et al. 2009; Molino, Childs et al. 2009; Briand et al. 2012). These studies suggest that the type of biocide and environmental conditions affected the composition of the bacterial and diatom communities. Moreover, only a few investigations have been reported on the long-term (>6 months) microbial fouling on biocidal coatings (Igic 1988; Jelcic-Mrcevic et al. 2006). None of these have used molecular methods for microbial identification. Since microbial fouling affects the performance of commercial biocidal coatings (Yebra et al. 2004; Schultz 2007;
Schultz et al. 2011), the quantitative and qualitative description of marine microbial fouling communities is of great interest to microbiologists and commercial anti-fouling coating manufacturers.

In this study, long-term (one year) microbial fouling on three types of commercial biocidal coatings (SPC, CDP, SPH) was investigated in terms of the abundance and diversity of organisms upon static immersion in the tropical marine environment of the Gulf of Oman. High throughput sequencing was used for identification of microorganisms. The hypothesis that distinct microbial fouling communities would develop on different types of biocidal coatings was tested.

**Materials and methods**

**Coating preparation**

Eleven commercial biocidal coating treatments were applied onto cleaned, degreased and abraded metal panels (15 × 28 cm) by airless spray at AkzoNobel’s Marine Coatings laboratory (AkzoNobel, International Paint Ltd, Felling, UK). All coating treatments were applied as a full coating scheme comprising a primer (Intershield 300®), tie coat (Intergard 263) and biocidal coating Intersmooth 100 SPC (ISM1); Intersmooth 7460HS SPC (ISM2); Intersmooth 7465HS SPC (ISM3); Intersmooth 360 SPC (ISM4); Intersmooth 365 SPC (ISM5); Intersmooth 460 SPC (ISM6); Intersmooth 465 SPC (ISM7); Interspeed 6200 (ISP1); Interspeed 6400 (ISP2); Interswift 655 (ISW1) and Interswift 6800HS (ISW2). The Intersmooth products are classified as SPC coatings, the Interswift products are classified as SPH coatings and the Interspeed products are classified as CDP coatings (Table 1). The nominal dry film thicknesses were 2 × 150 μm for the primer, 100 μm for the tie coat and 2 × 125 μm for the biocidal coating. These coating schemes are representative of those typically used on commercial vessels. Panels coated with the primer and tie coat scheme alone were maintained as the control treatments. After the whole scheme was applied, all coated panels were dried for several days at ambient temperature prior to dispatch to Oman for deployment. Each coating treatment including the control was prepared and tested in triplicate.

**Coatings deployment and sample collection**

All panels were attached to plastic pipes (80 cm long with an internal diameter of 3 cm) using cable ties (0.5 cm wide) through holes drilled on the uncoated area of each panel (1 cm from the top edge and 5 cm both from left and right edges). This was done in a randomized manner such that the three replicates of each treatment (coated with/without biocidal coating) were randomly allocated positions on each pipe. A plastic spacer (uncoated panels, 15 × 20 cm) was inserted between the control and the biocidal coating treatment panels. All the pipes with attached panels were deployed by ropes attached at both ends such that the panels were suspended vertically to the surface at a depth of 1 m. Panels were submerged for a period of one year (May 2011–April 2012) in the semi-enclosed Marina Bandar Rowdha (Muscat, Oman 23°34′55″ N, 58°36′27″ E).

At the end of the experiment, biofilms were scraped into sterile plastic containers using a sterile scalpel and immediately transferred to the laboratory on ice. Biofilms were collected from all panels. Since the control panels were heavily fouled by macrofouling species, only a minimal amount of biofilm (1–2 g) could be obtained from the control panels. All samples were stored at −20°C and processed within several weeks (see below).

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Table 1. Characteristics of the 12 coating treatments exposed (in triplicate) to biofouling for one year at a depth of 1 m in Marina Bandar Rowdha.

| Treatments | Coating(s) | Type of coating | Biocides                  |
|------------|------------|-----------------|---------------------------|
| Control    | Intershield 300 + Intergard 263 | Primer + Tie coat | Tralopyril + ZnPT         |
| ISM1       | Intershield 100 SPC | Self-polishing copolymer (SPC) | Cuprous oxide + CuPT     |
| ISM2       | Intersmooth 7460HS SPC | Self-polishing copolymer (SPC) | Cuprous oxide + CuPT     |
| ISM3       | Intersmooth 7465HS SPC | Self-polishing copolymer (SPC) | Cuprous oxide + ZnPT     |
| ISM4       | Intersmooth 360 SPC | Self-polishing copolymer (SPC) | Cuprous oxide + ZnPT     |
| ISM5       | Intersmooth 365 SPC | Self-polishing copolymer (SPC) | Cuprous oxide + ZnPT     |
| ISM6       | Intersmooth 460 SPC | Self-polishing copolymer (SPC) | Cuprous oxide + ZnPT     |
| ISM7       | Intersmooth 465 SPC | Self-polishing copolymer (SPC) | Cuprous oxide + ZnPT     |
| ISP1       | Interspeed 6200 | Controlled depletion polymer (CDP) | Cuprous oxide + zineb    |
| ISP2       | Interspeed 6400 | Controlled depletion polymer (CDP) | Cuprous oxide + zineb    |
| ISW1       | Interswift 655 | Self-polishing coatings (SPH) | Cuprous oxide + CuPT     |
| ISW2       | Interswift 6800HS | Self-polishing coatings (SPH) | Cuprous oxide + CuPT     |

Note: CuPT = copper pyrithione, ZnPT = zinc pyrithione.
Analysis of microfouling communities

The presence of macrofouling on the panels was visually evaluated. The total wet weight (± 0.001 g) of biofilms collected from the panels was determined using a balance. The bacterial counts within biofilms were estimated by staining 3 mg of biofilm with 4 µl of 4,6-diamidino-2-phenylindole (DAPI, Sigma, Munich, Germany) solution as previously described (Dobretsov & Thomason 2011). The number of bacteria in 20 randomly selected fields of view (0.031 mm²) was then counted using an epifluorescence microscope (Axiostar plus, Zeiss, Gottingen, Germany; magnification 1000×; λ_ex = 359 nm, λ_em = 441 nm). The biomass of photosynthetic organisms was analysed by the quantification of chlorophyll a. Biofilm samples (0.1–0.3 g) were extracted in 1 ml of 90% acetone (Thermo Fisher Scientific Inc., Waltham, MA, USA) in the dark for 24 h. The amount of chlorophyll a was determined spectrophotometrically according to Lorenzen (1967). Biofilm (3 mg) was gently disrupted on a clean glass slide using a sterile scalpel after adding 70 µl of autoclaved filtered (0.2 µm) seawater. The major genera of diatoms were identified by observing 25 randomly selected fields of view (0.19 mm²) using light microscopy (Axiostar plus, Zeiss; magnification 400×).

Pyrosequencing analysis

Biofilm samples (wet weight = 0.1 g) from each sample were subjected to DNA extraction using a Power Biofilm MoBio Kit (MOBIO, Hamburg, Germany) following the manufacturer's instructions. DNA extracts from three replicated samples of the same treatment were pooled together in equal amounts. Finally, 11 biocidal coatings and one control DNA extract were obtained. DNA extracts were submitted to the Research and Testing Laboratory (RTL, Lubbock, TX, USA). The primers used for the amplification of 16S rRNA were 28F (GAGTTTGATCCTGCTCAG) and 519R (GTNNTACNGCGGCKGCTTG), generating a fragment size of c.491 bp. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed according to the RTL protocols (www.researchandtesting.com). Sequences were trimmed; low quality ends and tags were removed and were checked for chimeras using custom software (Dowd et al. 2008) and the Black Box Chimera Check software B2C2 (http://www.researchandtesting.com/B2C2.html). Sequences < 300 bp were excluded from further analysis and the rest were checked for high quality based on criteria utilized by the Ribosomal Database Project version 9 (Cole et al. 2009). These sequences were analysed and taxonomically classified using a distributed BLASTn. NET algorithm (Dowd et al. 2005) against a database of high quality 16S rRNA gene sequences obtained from NCBI. The outputs were compiled and validated using taxonomic distance methods (Dowd et al. 2008). For data analysis, Mothur software version 1.31.2 (Schloss et al. 2009) was used. Rarefaction curves, and operational taxonomic unit (OTU) number based on 97% cutoff were calculated. The OTU richness was determined by Chao1 and Shannon diversity indices.

Statistical analysis

One-way ANOVA was used to test the effect of biocidal coatings on the total wet weight of biofilm, the density of bacteria and the concentration of chlorophyll a using Statistica 11 (Statsoft, Tulsa, OK, USA). Before the analysis, the normality of the data was verified (Zar 1998). The post hoc Tukey honestly significant difference (HSD) test was used to test for significant differences in the total wet weight of biofilm, the density of bacteria and the concentration of chlorophyll a among the biocidal coatings. In all cases, the threshold for significance was 5%. Single linkage cluster analyses were used to group the microbial communities in terms of the relative abundance (%) of classes of bacteria and the presence or absence of major species of diatoms, respectively. Data were square root transformed for the construction of a similarity matrix. The effect of biocidal coating treatments on the bacterial communities was analysed using analysis of similarity (ANOSIM) and similarity percentage (SIMPER) procedure (PRIMER software, Plymouth Marine Laboratory, Plymouth, UK). These analyses were based on the Bray–Curtis similarity index (Clarke 1993).

Results

Fouling microorganisms

After one year, visual observations revealed that all the biocidal coatings were 100% free of macrofouling and only microbial biofilms were detected. All the biocidal fouling control coatings significantly affected the formation of biofilms (Figure 1A; ANOVA, p < 0.0001). The lowest biofilm wet weight (9 mg cm⁻²) was recorded on coating ISM2 while the highest (25 mg cm⁻²) was recorded on coating ISP1. There was also a significant influence of biocidal coatings on the total bacterial density in biofilms (Figure 1B; ANOVA, p < 0.0001). Bacterial density ranged from 43,873 ± 20,445 to 275,932 ± 70,690 cells cm⁻² (± SD), with the highest counts detected on coating ISM1 and the lowest on coating ISM7. However, there was no significant effect of the biocidal coatings on the concentration of chlorophyll a in the biofilms developed on all coatings (Figure 1C; ANOVA, p = 0.13).

A total of eight species of diatoms (Nitzschia sp., Navicula sp., Navicula directa, Amphora sp., Cylindrotheca sp., Mastogolia sp., Licmophora sp. and Amphiprora sp.) were found in the biofilms collected from all panels.
Diatom communities formed on the control were different from those developed on the biocidal coatings. On the biocidal fouling control coatings, the highest species richness (six species) was observed on coatings ISM5 and ISP2, while the lowest (three species) was found on coating ISM1 (Table S1). Cluster analysis demonstrated that diatom communities formed on ISW2 and ISM1 were different from those on the other coatings (Figure 2). There was no clear difference between diatom communities formed on the different types (CDP, SPC and SPH) of coatings.

**Pyrosequencing of bacterial communities**

A total of 48,628 sequences of 16S rRNA genes with an average size of 450 bp were obtained from all samples. The control panel showed the highest number of OTUs (506), followed by the SPC coating ISM7 (422) (Table 2), while the lowest number of OTUs (29) was detected in biofilms from ISM1 (Table 2). The number of OTUs on CDP coatings was 1.3–1.8-fold higher than on SPH coatings. A similar pattern was also observed for the Chao1 and Shannon indexes (OTU richness); the lowest diversity was observed in ISM1 biofilms and the highest in the control (Table 2). In the case of ISM1, the rarefaction curves reached saturation level at 400–500 sequences, which indicated very low species diversity in this biofilm (Figure S2). In contrast, the curves of other coatings and especially those of the control did not level off at the 97% cut-off level, and more sequences were apparently needed to cover the whole community diversity.

In total, 31 bacterial classes were detected in the biofilms which developed on all the coatings. Sequences from the phylum Cyanobacteria (17–51% of total sequences) and classes Flavobacteria (10–42%), Alphaproteobacteria (17–56%) and Gammaproteobacteria (2–32%) accounted for > 90% of total sequences in each biofilm (Figure 3). Cluster analysis showed that distinct bacterial communities were formed on ISM1 (SPC coating), ISW1 (SPH coating), ISP1 and ISP2 (CDP coating), as well as on the control coating (Figure 3). SIMPER analysis revealed that Flavobacteria accounted for 16% and 17% of the dissimilarity between communities developed on the control and ISM (SPC) coatings and on the control and ISW (SPH) coatings, respectively (Table 3). In contrast, Alphaproteobacteria contributed to ca.14% of the dissimilarity between control and ISP (CDP) coatings. However, in pairwise comparisons within the three types of coatings, members of the phylum Cyanobacteria contributed to 13% and 20% of the dissimilarity between control and ISP (CDP) coatings.
bacterial communities which developed on ISP and ISM coatings and on ISP and ISW coatings, respectively (Table 3). Alphaproteobacteria contributed to 14% of the dissimilarity between bacterial communities on ISM and ISW coatings.

Sequences belonging to the cyanobacterial genus *Acaryochloris* constituted 20–48% of total sequences on all coatings, except for ISM1 where it was not detected (Figure 4). Instead, the major cyanobacterial sequences in the ISM1 biofilm belonged to the genus *Synechococcus* (20% of the total sequences). Sequences related to *Leptolyngbya* contributed to 16–24% of the total sequences in ISM2 and ISM7 samples (Figure 4). Other cyanobacterial sequences belonging to the genera *Cyanothecae, Phormidium, Anabaena* and *Oscillatoria* were detected but at a much lower abundance.

Table 2. Calculated diversity indices based on 454 pyrosequencing of the bacterial communities in the biofilms on the control and all coating treatments.

| Sample | Number of sequences | No. of OTUs | Chao 1 (Lower/upper) | Shannon |
|--------|---------------------|-------------|----------------------|---------|
| Control | 3,042 | 506 | 918 (801/1,080) | 4.58 |
| ISM1 | 2,157 | 29 | 47 (33/110) | 2.41 |
| ISM2 | 5,670 | 385 | 728 (616/895) | 3.66 |
| ISM3 | 3,229 | 288 | 446 (388/539) | 3.96 |
| ISM4 | 4,976 | 277 | 374 (335/439) | 3.57 |
| ISM5 | 3,265 | 229 | 342 (294/425) | 3.35 |
| ISM6 | 4,484 | 331 | 695 (566/896) | 3.89 |
| ISM7 | 6,169 | 422 | 669 (590/784) | 4.15 |
| ISP1 | 3,175 | 280 | 463 (395/573) | 3.04 |
| ISP2 | 4,745 | 315 | 549 (466/677) | 3.32 |
| ISW1 | 2,598 | 174 | 233 (206/281) | 2.82 |
| ISW2 | 5,118 | 207 | 303 (259/384) | 3.26 |

Figure 3. Heat map showing the relative abundance (%) of six major classes of bacteria present on all coating treatments (control, ISM1, ISM2, ISM3, ISM4, ISM5, ISM6, ISM7, ISP1, ISP2, ISW1 and ISW2).
Table 3. The contribution of particular classes of bacteria to total dissimilarity (as percentages) between the bacterial communities on the control and the coated panels using SIMPER analysis.

| Class/Phylum        | Contribution (%) | Comparison with control | Comparison within coatings |
|---------------------|------------------|------------------------|--------------------------|
|                     | ISM   | ISP   | ISW   | ISM:ISP | ISM:ISW | ISP:ISW |
| Flavobacteria       | 16.69 | 12.93 | 15.57 | 9.61     | 9       | 8.07    |
| Alphaproteobacteria | 12.91 | 13.95 | 8.24  | 9.38     | 13.86   | 11.51   |
| Cyanobacteria       | 6.28  | 9.24  | 9.48  | 13.18    | 11.65   | 20.15   |
| Gammaproteobacteria | 8.82  | 6.89  | 4.82  | 12.93    | 9.34    | 6.73    |
| Actinobacteria      | 6.95  | 6.68  | 8.15  |          |         |         |
| Verrucomicrobia     | 5.99  | 6.8   | 6.23  |          |         |         |
| Deinococci          | 6.47  | 5.93  | 6.04  | 5.78     | 6.46    |         |
| Acidobacteria       |       | 6.44  | 6.11  | 7.09     | 6.49    |         |
| Cytophagia          |       |       |       |          |         |         |
| Planctomycetia      |       |       |       |          |         |         |
| Deltaproteobacteria |       |       |       |          |         |         |
| Others              | 42.36 | 43.51 | 35.11 | 35.81    | 32.58   | 42.45   |

Note: Values in bold indicate highest contribution (%). Bacteria contributing < 5% are not shown.

Bacteria belonging to the bacterial genus *Maritimimonas* were present on all coatings except ISM1 (Figure 4). Sequences belonging to this genus constituted 27–20% of the total sequences on coatings ISM4, ISM5, ISM6, ISP2, and ISW2. In contrast, 16.6% of total sequences in coating ISM1 were related to the bacterial genus *Flavobacterium* and 38% of total sequences in the ISW1 biofilm belonged to the genus *Lutibacterium*. Biofilms on coating ISM1 were characterized by several unique bacterial genera, such as *Candidatus Pelagibacter*, *Amaricococcus*, *Neptuniibacter*, *Photobacterium*, *Psychrobacter* and *Clostridium* (Figure 4). Similarly, the genera *Psychroserpens* and *Gilvibacter* were found only on coating ISM4.

**Discussion**

The commercially available biocidal fouling control coatings tested in this study exhibited high efficiency in preventing macrofouling and significantly inhibited, but did not completely prevent, microfouling after static exposure for a period of one year in a tropical marine environment. The highest amount of biofilm as well as total bacterial density were found on the CDP coatings (ISP1 and ISP2), followed by the SPH coatings (ISW1 and ISW2) and the SPC coatings (ISM1, ISM2, ISM3, ISM4, ISM5, ISM6, and ISM7). This apparent relative ranking is in line with the general performance expectations of the types of coatings tested in this study. The lowest amount of biofilm on ISM2 and the lowest density of bacteria on ISM7 could be attributed to the highly controlled biocide leaching rate for SPC coating technology in comparison with SPH and CDP coatings over extended periods (Finnie & Williams 2010). This result is consistent with earlier observations revealing lower biomass values on SPC coatings in comparison with the control after immersion for one year in the East Adriatic Sea (Jelic-Mrcelic et al. 2006).

In this study, for the first time the composition of microbial biofilms on antifouling coatings was investigated via the pyrosequencing of bacterial 16S genes. Bacteria belonging to the classes Alphaproteobacteria, Flavobacteria and the phylum Cyanobacteria were the most dominant on all coatings (Figure 3). Previous studies indicated the dominance of Alphaproteobacteria in marine biofilms on different substrata (Chung et al. 2010; Chen et al. 2013; Dobretsov et al. 2013). Bacteria belonging to the classes Alphaproteobacteria, Gammaproteobacteria, Firmicutes and Bacteroidetes have been found on the hulls of ships coated with cuprous oxide based coatings (McNamara et al. 2009). While the presence of Deltaproteobacteria was reported on copper-based biocidal coatings (Chen et al. 2013), this class, mostly represented by sulphate- and sulphur-reducing anaerobic bacteria, was absent in the present investigation. It may be speculated that Deltaproteobacteria were outcompeted by other groups or absent due to a high abundance of cyanobacteria, which created fully oxygenated biofilms.

The presence of the cyanobacterium *Acaryochloris marina* containing unique photopigments such as chlorophyll *d* (Miyashita et al. 2003) and *α*-carotene (Takaichi et al. 2012) has been reported for the first time on all biocidal coatings, except ISM1, in the present study. Chlorophyll *d* facilitates oxygenic photosynthesis of this cyanobacterium with near-infrared light (Behrendt et al. 2011). The genus *Acaryochloris* has been previously found in diverse habitats including biofilms on colonial ascidians (Martínez-García et al. 2011), inside tissues of didemnid ascidians (López-Legentil et al. 2011), underneath crustose coralline algae (Behrendt et al. 2011), as well as in the waters of tropical reefs (Mohr et al. 2010) and saline and
freshwater lakes (Kashiyama et al. 2008). However, there was no species-specific relationship between the various phylotypes of Acaryochloris and marine invertebrates including didemnid ascidians and a sponge (Ohkubo & Miyashita 2012). The distribution of Acaryochloris, its ecological significance and the role of chlorophyll d-photosynthesis in the biosphere remain largely unknown (Behrendt et al. 2011; Loughlin et al. 2013).

The current study revealed that species of the heterotrophic bacterium Maritimimonas rapanae found on all coatings except ISM1 were highly abundant on selected SPC coatings (ISM4, ISM5 and ISM6) and one SPH coating (ISW2). This bacterium, originally isolated from the veined rapana whelk (Rapana venosa) collected in South Korea (Park et al. 2009), represents a novel species in a new genus of the family Flavobacteriaceae. To date, there are no reports of the existence of this bacterium on biocidal coatings.

The abundance of photosynthetic organisms did not show any significant (ANOVA, HSD, p > 0.1) differences among the various coatings. This result could be due to the high abundance of Cyanobacteria on the
panels (Figures 1 and 3). The cyanobacterial species *Chroococcus minor* and *Synechococcus* sp. are not sensitive to high concentrations of copper (\(>1,200 \mu g \text{ L}^{-1}\)) and are moderately sensitive to ZnPT and CuPT (\(>22 \mu g \text{ L}^{-1}\)) in comparison with other marine species (Bao et al. 2011). Different diatom communities were formed on the coatings. The diatom *Amphora* sp. was not found on the ISM1 coating, while *Nitzschia* sp. was not observed on the SPH coatings (ISW1 and ISW2), and *Licmophora* sp. was absent on all biocidal coatings (Table S1). In contrast, the presence of diatoms belonging to the genera, *Nitzschia*, *Navicula*, *Amphora* and *Licmophora* was previously reported on antifouling coatings (Callow 1986; Cassé & Swain 2006; Pelletier et al. 2009; Briand et al. 2012; Zargiel & Swain 2014). These results could indicate different sensitivities of diatom species towards biocides in antifouling coatings (Molino, Campbell et al. 2009).

Distinct bacterial community structures on different types of coating have been found, which may be attributed to the differences in the composition of the biocides present in these coatings. For example, the unique bacterial community structure in the biofilm on coating ISM1 could reflect the replacement of the cuprous oxide/pyrithione combination with tralopyril and zinc pyrithione. The presence of the bacterial genera *Acaryochloris*, *Maritimumonas*, *Muricauda* and *Erythrobacter* on all coatings except ISM1 may reflect the broad spectrum of antibacterial activity of the combination of tralopyril with zinc pyrithione (Kempen 2012). The presence of this metal-free biocide and/or co-biocide (CuPT) may have induced a selective chemical environment for the growth and development of specific genera of bacteria, such as *Neptunibacter*, *Photobacterium*, *Psychrobacter*, *Amaricoccus*, *Candidatus Pelagibacter* and *Clostridium*, found only on coating ISM1 (Figure 4). However, the efficiency of tralopyril against bacterial fouling in the current study is uncertain. Although coating ISM1 had the highest bacterial density it had the lowest number of OTUs in comparison with other coatings. Hence, further experiments are required to investigate the potential efficacy of tralopyril and tralopyril/co-biocide combinations on managing long-term bacterial fouling in the marine environment.

The differences in the formation and composition of the biofilms which developed on the coatings tested in the current study can be attributed to the coating type, how it functions and its biocide composition. In particular, the polishing rate and biocide delivery rate are known to vary for different coating types (Bressy et al. 2010; Finnie & Williams 2010). Additionally, established biofilms could lead to significantly lower Cu\(^{2+}\) release rates from a different series of commercial coatings (Valkirs et al. 2003; Yebra et al. 2006b). It is therefore not surprising that the coatings in the present study show some differences in the quantity and species diversity of the biofilms that developed on them. Indeed, given the differences and the broad range of coatings tested, it is perhaps surprising that there are not more substantial differences in the biofilms. However, it should be remembered that the coatings used in the study are designed for use on commercial shipping whose activity profile typically includes alternating periods at sea and in port. While some ships may experience extended periods of inactivity, eg during newbuilding, outfitting or if laid up, these biocidal coatings would ordinarily not be the subject to one year exposure under static conditions. It would be expected that a more differentiated performance among the different coatings would manifest under a more realistic and representative test regime for commercial shipping activity.

In conclusion, this study demonstrates that biocidal coatings representative of the three main historic types of biocidal fouling control coatings prevented macrofouling and significantly resisted the formation of microfouling when immersed under static conditions in a tropical marine environment for one year. Longer term studies under dynamic conditions encompassing a wider range of coatings, including fouling-release products and the newer biocidal coatings that have been introduced since the present study commenced, would help elucidate the link between coating type, function and composition on their ability to control fouling in real world scenarios.

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