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Research Article

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Differential early dynamic process of marine eukaryotic microbial community on anti-biofouling and biofouling-enhancing micro/nano surfaces

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ABSTRACT

In this study, marine eukaryotic microbial community was analyzed in the context of its complexity and dynamic process in the early stage of biofouling. Samples were collected from five different surfaces: the control group (coating CG), 0.2% Silicium (Coating A), 2% Carbon nanopowder (Coating B), 0.2% Graphite (Coating C) and 2% Silicium plus 2% Carbon nanopowder (Coating F). These coatings were sea-tested for 11 months for their anti-biofouling performance. Coating A and B don't have anti-biofouling ability just like coating CG, while coating C and F have excellent anti-biofouling capacity. Single stranded conformation polymorphisms (SSCP) was employed to analyze diversity level and dynamic changes of marine fouling eukaryotic communities adhering to different coatings during different sampling time (day 2, 4, 6, 9 and 14). The results indicated that composition, distribution and diversity level of marine fouling eukaryotic microbial community presented obviously differences as sampling time and coating surfaces changed. Coatings with good anti-biofouling performance tend to have low diversity level and different adhering dynamics of marine fouling eukaryotic microbial community.

Key words: micro/nano materials; anti-biofouling; biodiversity; eukaryotic; microbial community

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INTRODUCTION

Biofouling is a severe problem in marine industry and navies around the world. In marine environment, submerged surfaces are colonized by biofoulers, including microfoulers (such as marine bacteria, algae, fungi, and protozoa) and macrofoulers (such as barnacles, oyster, bryozoans, and tubeworms) [1]. It has been estimated that fuel consumption would constantly increase if no effective anti-biofouling measures were adopted [2]. However, many widely used anti-biofouling compounds like tributyl tin (TBT), copper or organic compounds are toxic pollutants in aquatic environments [3]. There is a badly need to develop economically feasible and environmentally-friendly methods for biofouling control. The exploitation and utilizition of nanoparticle-based coatings represents a promising perspective for development of non-toxic control technologies through coatings that can prevent or slow the growth of some macrofouling organisms. So far, many studies have focused on the exploitation and utilization of metal or metal oxide micro/nano anti-biofouling coatings, such as silver and copper nanoparticles [4-6], nanoparticles made of titanium dioxide(TiO2), magnesium oxide (MgO), copper oxide (CuO) and zinc oxide (ZnO) [7]. Much less research has been done on nonmetallic micro/nano anti-biofouling coatings. Silicon dioxide (SiO2) and highly pure single-walled carbon nanotubes (SWNT) have been reported to have strong anti-biofouling activity [8-9].

Marine fouling eukaryotic organisms fulfill a range of crucial ecological functions and play an important role in the early stage of biofouling in marine ecosystems. Marine fouling eukaryotic organisms as a whole may be helpful to evaluate anti-biofouling property of different coating surfaces. Marine diversity is currently one of the most studied topics in ecology especially under the framework of marine environmental changes. Understanding diversity level and dynamic changes of marine fouling eukaryotic organisms adhering to surfaces of anti-biofouling coatings is of

great importance for studying the anti-biofouling mechanism. Culture-independent techniques provide ways for evaluation of diversity level among different microbial communities. The application of DNA-based technologies, such as the polymerase chain reaction (PCR), fluorescence in situ Hybridization(FISH), denaturing gradient gel electrophoresis (DGGE), temporal temperature gradient gel electrophoresis (TTGE), single stranded conformation polymorphisms (SSCP) and others facilitates investigation of the diversity, dynamics and identity of microbes in marine realms [10-11]. These methods are entirely molecular, thereby providing a potential link between ecological processes and the organisms involved. The variability and application of culture-independent tools has imposed a major impact on marine microbiology. Diversity assessments by using molecular approaches offer the possibility to characterize marine fouling eukaryotic organisms accurately. Nevertheless, current study on marine fouling fungi biodiversity is very limited. To date, few studies have focused on marine fouling eukaryotic microorganisms adhering to anti-biofouling coatings. Most studies have focused mainly on description of diversity levels of fouling prokaryotic organism community on panels or on ship hulls [12-15].

The aim of this study was to assess the anti-biofouling property of four nonmetallic micro/nano coatings, analyzing diversity levels and dynamic changes of marine fouling eukaryotic organisms on their surfaces at an early fouling stage using SSCP technology.

EXPERIMENTAL SECTION

Marine adhesion test and sampling. The immersion site named 'Xiaoshi' Island is located on the eastern coast of Weihai (N37˚31′51″; E″121˚58′19″), China. Marine adhesion test using steel panels (10cm×10cm at 1.5 meter depth, each coating repeated for 3-4 times) lasted for 11 months from May 2011 to April 2012. Selected coatings were freshly made for immersion and sampled from November 7th to November 19th, 2012, namely Nov7th(2d), Nov9th(4d), Nov11th(6d), Nov14th(9d) and Nov19th(14d). The average temperature of sea water during the sampling period was about 12℃. The steel panels were washed with sterile ddH2O. Biofilm sampling was undertaken by scraping the coating surfaces with brushes. All samples were preserved in -80℃ refrigerator for further analysis.

Micro/nano coatings fabrication. Three micro/nano materials were purchased from Sigma-Aldrich, and they were Silicon powder (Cat.No. 215619, 99% purity, 325 mesh), Carbon nanopowder (Cat.No. 633100, <30nm, 99% purity) and Graphite powder (Cat.No. 282863, <20 micron). The control coating was the commonly used iron oxide red primer, while the other four coatings were fabricated through addition and well-mixing of different amounts of the three non-nonmetallic micro/nano materials into the control coating (Table 1). All the coatings were painted as a film on steel panels with the same thickness (2mm) and dried for three days at the room temperature. The steel panels were then immersed into the sea water at 1.5m depth over different durations.

ITS rDNA amplification. Universal primers ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3') and ITS5 (5'- GGA AGT AAA AGT CGT AAC AAG G-3') used for amplification of ITS rDNA genomic sequences were synthesized from Sangon (Shanghai). All PCR reactions were performed at 12ul volume using NPK02 kit (GREDBIO) on a T-Gradient Thermal device (TAKARA) with an initial denaturation step at 94℃for 3 min, followed by 43 cycles of 30s at 94℃, 40s at 56℃, and 40s at 72℃and a final elongation step at 72℃for 2min. PCR products were detected by 1.2% agarose gel electrophoresis, and visualized in WD-9413C gel imaging analysis system (LiuYi, Beijing). All other reagents (unless noted) were purchased from Sangon.

SSCP gel electrophoresis. SSCP electrophoresis was performed at 4°C with 8% polyacrylamide gels (acrylamide: bisacrylamide 29:1) submerged in 0.5×TAE buffer with the DYCZ-24DN apparatus (LiuYi, Beijing) electrophoresis system. PCR products was mixed (1:1) with gel loading buffer (95% formamide, 0.25%bromphenol blue, 0.25% xylene cyanol) in a final volume of 10 ul. After incubation for 10min at 98℃, the sample was immediately cooled on ice for 15min before loaded and separated at 300 V for 6 h. The gel was then silver stained and SSCP fingerprints were recorded using digital camera PL2000 (SamSung) for further analysis.

Data analysis. Scanned gels were analyzed to understand the SSCP community profiles using Quantity One Software from Bio-Rad (version 4.6.2). Cluster analysis of profiles was performed and a dendrogram was constructed using unweighted-pair group method arithmetic mean (UPGMA) method. Peak Intensity values were used for comparison of bands within profiles to determine the diversity indices by considering each band analogous to a single species and taking band density as equivalent to species richness. Band positions and intensity were performed to obtain data matrices. On the basis of the data matrices, diversity of marine fouling eukaryotic microbial community was analyzed by using Biodap software. Furthermore, diversity indices (Shannon-Wiener diversity index, Simpson Index and evenness index) were employed to estimate the diversity level of the marine fouling eukaryotic communities.

RESULTS AND DISCUSSION

Marine adhesion test results. As can be seen from Figure 1, the commercially available paint coating CG (Control Group), coating A as well as coating B have suffered serious fouling and corrosion triggered by biofoulers. In contrast, coating C and coating F have ideal anti-biofouling effect with few macrofoulers adhered on surfaces, implying that some nonmetallic micro/nano materials as additives can greatly enhanced anti-biofouling effect. Interestingly, the additions of Silicium and Carbon nanopowder separately have no anti-biofouling effect, while their combinations have significant biofouling-resistant effect.

Table 1 Anti-biofouling effect of five coatings

Coating	Micro/Nano materials	Anti-biofouling effect	Biofouling-enhancing effect
CG	-	-	+
A	Silicium (0.2%)	-	+
B	Carbon nanopowder (2%)	-	+
C	Graphite powder (0.2%)	+	-
F	Silicium (2%) & Carbon nanopowder (2%)	+	-

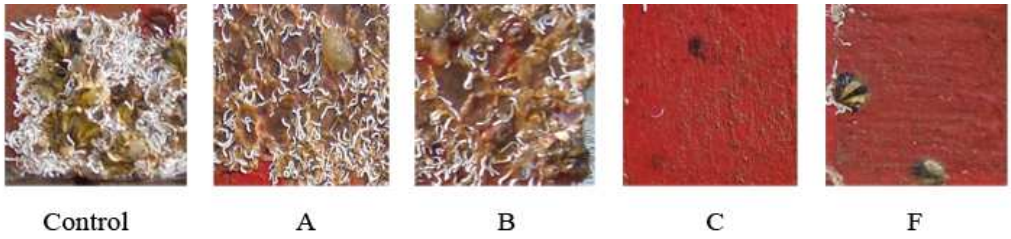
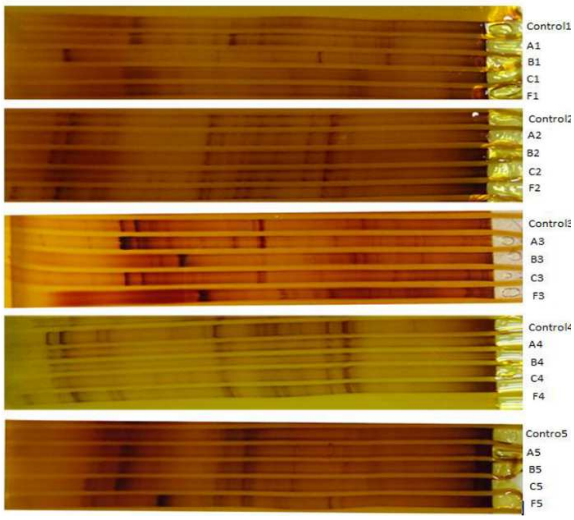


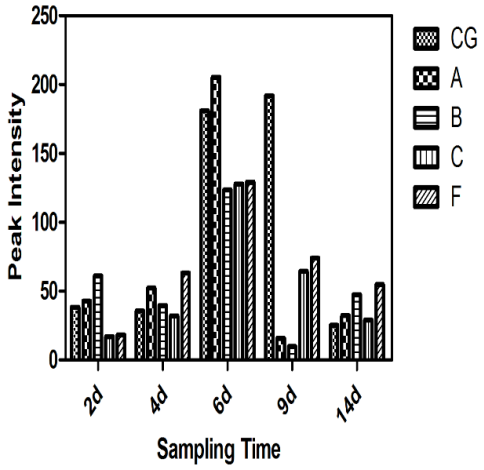
Figure 1 Anti-biofouling performance of micro/nano coatings after an exposure to marine environment for 11 months

Community analysis by SSCP profile. With the aim to objectively evaluate anti-biofouling performance of nonmetallic micro/nano coatings and explore possible molecular mechanism, SSCP technology was employed to evaluate diversity level and dynamic changes of marine fouling eukaryotic communities. SSCP gave diverse banding patterns as illustrated in Figure 2A. According to SSCP fingerprinting, biodiversity level of marine fouling eukaryotic microbial community adhering to different coating surfaces at the same sampling time presented clear differences; different community succession phenomenon was seen among different samples, demonstrating that marine fouling eukaryotic microbial communities were under dynamic changes. Besides, the peak intensity of the SSCP bands can be appropriately considered to reflect the amount of ITS fragments amplified. The graphical representation of peak intensity was shown in Figure 2B, which illustrated values of peak intensity undergone fluctuations, peaking at 6d and then gradually tends to stay stable. According to Figure 1 and Figure 2B, it can be estimated that nonmetallic micor/nano coatings with good anti-biofouling property tends to have lower species richness of marine fouling eukaryotic microbial community, which implied that the addition of nonmetallic micro/nano material influenced or changed community succession of marine fouling eukaryotic microbial community. The observed anti-biofouling effects may have certain relationships with ability to change or lower fouling species richness of eukaryotic microbial community.

Clustering results (Data not shown) indicated that marine eukaryotic microbial communities adhering to surfaces of different coatings at the same sampling period exist significant divergence from each other. Samples taken from surface of coating A, coating B and coating CG displayed similar features, thus most samples clustered into the same category. Nevertheless, samples of the coating C and coating F varied from those compared to coating CG , thereby gathering for another category, which indicated that the addition of nonmetallic micro/nano materials change marine eukaryotic microbial community on surfaces of coating, coatings with good anti-biofouling property have strong ability to make marine eukaryotic microbial community change happen.



(A)



(B)

Figure 2 Basic SSCP data

(A)Fngerprints of ITS rDNA. Samples were divided into five groups (coating CG, coating A, coating B, coating C and coating F). Each group has five sampling points, corresponding to day 2, day 4, day 6, day 9 and day 14. (B) Total peak intensities of ITS DNA bands for different coating samples from 2d, 4d, 6d, 9d and 14d, respectively.

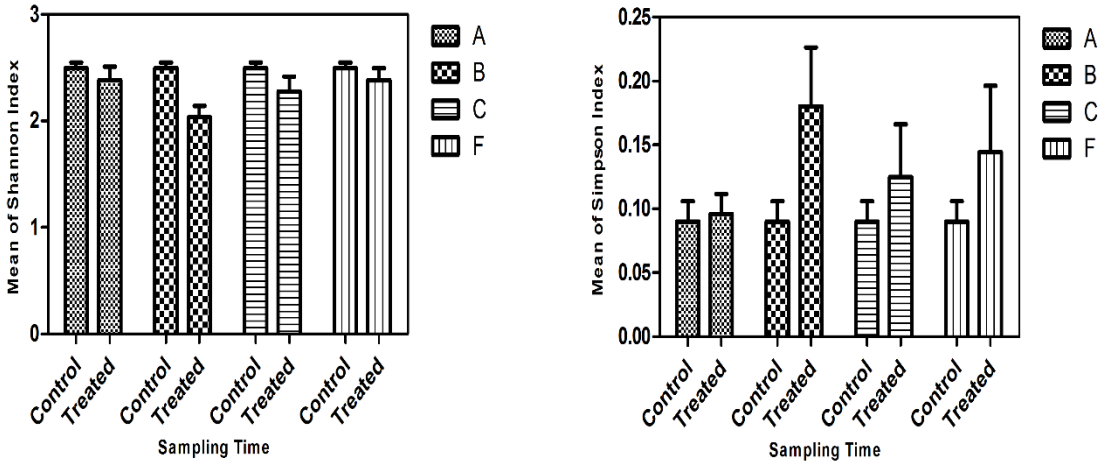


Figure 3 Comparison of mean diversity indices before and after adding of nonmetallic micro/nano materials

Diversity indices were calculated to further analyze and quantify biodiversity and dynamics change of marine fouling eukaryotic community, as is shown in Table 2. Shannon-Wiener index was used for evaluation of diversity and evenness level of marine fouling eukaryotic community, whereas Simpson index reflected changes of advantage species [16-17]. Figure 3 compared mean of Shannon-Wiener index and Simpson index changes. It can be estimated that the addition of nonmetallic micro/nano materials resulted in slightly decrease of Shannon-Wiener index while Simpson index was seen on the rise, indicating that diversity level of marine fouling eukaryotic community was lowered and dominant species were increased, thereby changing evenness level and succession process of marine fouling eukaryotic microbial communities and accelerating the formation of the climax community. As a result, diversity indices may be used as evaluation standard for anti-biofouling performance of nonmetallic micro/nano coatings from molecular ecology perspective. Marine fouling eukaryotic microbes as a significant role of marine ecosystems may fulfill a range of crucial ecological functions participation in the early stage of biofouling. Diversity level of marine fouling eukaryotic microbial community is extremely important to anti-biofouling property testing of anti-biofouling coatings. Coatings with ideal anti-biofouling effect tend to have the ability to lower diversity level of marine fouling eukaryotic microbial community and maintain the stability of community structure.

Table 2 Molecular ecology parameters of marine eukaryotic microbial community

Serial number	Sample Name	Shannon-Wiener Index	Simpson diversity Index	Evenness Index	Species richness	Total abundance
	CG1	2.62	0.072	0.906	18	38.21
	A1	2.66	0.055	0.959	16	42.68
2d	B1	2.23	0.168	0.804	16	60.88
	C1	2.21	0.065	0.96	10	17.02
	F1	2.28	0.072	0.918	12	18.14
	CG2	2.47	0.085	0.891	16	35.48
	A2	2.32	0.12	0.857	15	52.24
4d	B2	2.14	0.147	0.834	13	39.43
	C2	2.53	0.074	0.893	17	32.07
	F2	2.42	0.11	0.854	17	62.99
	CG3	2.33	0.141	0.765	21	180.94
	A3	2.52	0.124	0.773	26	205.4
6d	B3	2.22	0.186	0.754	19	123.5
	C3	2.26	0.164	0.768	19	127.72
	F3	2.13	0.346	0.752	17	129.05
	CG4	2.47	0.106	0.834	19	191.77
	A4	1.92	0.119	0.923	8	15.75
9d	B4	1.91	0.057	0.981	7	9.61
	C4	1.8	0.27	0.682	14	64.2
	F4	2.8	0.061	0.935	20	73.92
	CG5	2.59	0.045	0.956	15	25.14
	A5	2.5	0.062	0.947	14	32.25
14d	B5	1.67	0.342	0.633	14	47.39
	C5	2.58	0.051	0.953	15	28.94
	F5	2.28	0.132	0.842	15	54.64

CONCLUSION

In this study, the anti-biofouling property of several nonmetallic micro/nano coatings was evaluated by marine adhesion test. The possible anti-biofouling mechanism was preliminarily explored by using SSCP approach. Results showed that coating C (Graphite powder as additive) and coating F (Silicium and Carbon nanopowder as additives) have decent anti-biofouling effect. According to SSCP analysis, it can be postulated that possible anti-biofouling mechanism might be in that addition of nonmetallic micro/nano materials (combinations) could cause changes of surface morphology features of anti-biofouling coatings, lowering diversity level and changing succession dynamics of marine eukaryotic microbial community on them, eventually leading to the inhibition of marine biofouling.

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