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Reconciling the Conflict between Optical Transparency and Fouling Resistance with a Nanowrinkled Surface Inspired by Zebrafish's Cornea

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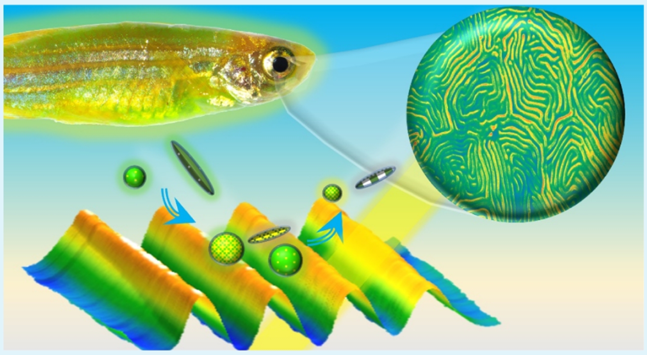

ABSTRACT: Surface topography has been demonstrated as an effective nonchemical strategy for controlling the fouling resistance of a surface, but its impact on optical transparency remains a barrier to the application of this strategy in optical materials. To reconcile the conflicting effects of surface topography on optical transparency and fouling resistance, here we study the optical properties and antifouling performance of nanowrinkled surfaces inspired by the corneal surface of zebrafish (*Danio rerio*). Experimental and numerical analyses demonstrate that a good compromise between optical transparency and antifouling efficacy can be achieved by wavy nanowrinkles with a characteristic wavelength of 800 nm and an amplitude of 100 nm. In particular, the optimal wrinkled surface under study can reduce biofouling by up to 96% in a single-species (*Pseudoalteromonas* sp.) bacterial settlement assay in the laboratory and 89% in a field test while keeping the total transmittance above 0.98 and haze below 0.04 underwater. Moreover, our nanowrinkled surface also exhibits excellent resistance against contamination by inorganic particles. This work provides a nonchemical strategy for achieving the coexistence of optical transparency and fouling resistance on one single material, which implies significant application potential in various optical devices and systems, such as antibacterial contact lenses and self-cleaning solar panels.

KEYWORDS: *transparency, fouling, surface engineering, bioinspiration, zebrafish*

INTRODUCTION

Optical surfaces applied in harsh environments are prone to the accretion of foreign matters, including organic and inorganic molecules, particles, bacteria, and viruses, and even sessile animals, leading to functional and economic detriments in a broad spectrum of applications. For instance, healthcare products[1] such as endoscopic cameras and contact lenses tend to be colonized by bacteria, resulting in high infection risks to patients and users. The attachment and proliferation of sessile animals in the ocean, such as barnacles and tubeworms, on underwater cameras and submarine windows cause material deterioration and loss of vision (Figure S1), ruining the optical functionality of the concerned devices.[2] Desert solar farming, an emerging industry for collecting solar energy, suffers from the accumulation of sand, dust, and other inorganic particles on solar panels, which drastically reduces the efficiency of energy conversion.[3] Controlling the accretion and fouling of other matters on these optical surfaces is challenging since the potential solutions to preventing fouling tend to interfere with optical transparency.

To reconcile the conflict between fouling resistance and optical transparency, different strategies have been proposed.[4−10] For example, chemical coatings were applied to reduce fouling attachment with no significant sacrifice of the optical transparency;[6,9] however, the compositions of these coatings might be chemically active[11] and may bring adverse side effects to the potential users. For example, some toxic chemical compounds have been shown to cause drastic alterations to ecosystems such as physiological alterations on mollusks and fishes[12,13] and broad waterway contaminations.[14] Topographical modifications such as the inclusion of micro- and nanotopographical features have been confirmed as an effective and environmentally friendly method to enhance the fouling resistance of surfaces,[15,16] but their influence on the optical properties is not well understood. A film with nanowires deposited on the surface achieved superior resistance to marine fouling and acceptable transmittance of light, but its high degree of haze may affect its visual quality.[7]

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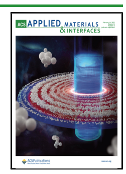

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The degradation and environmental risk of camptothecin, a promising marine antifoulant

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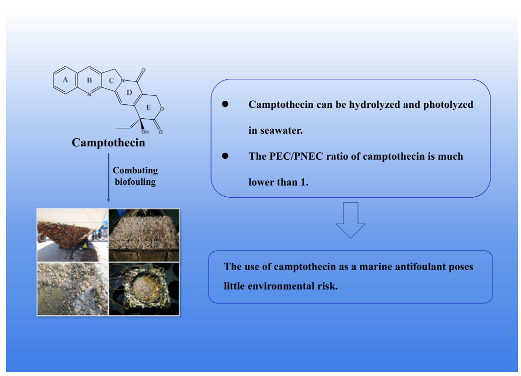
HIGHLIGHTS

- The antifouling active camptothecin is degradable in the marine environment.
- The fastest degradation pathway for camptothecin in seawater is photodegradation.
- The PEC/PNEC ratio of camptothecin is much lower than 1.
- The use of camptothecin as a marine antifoulant poses little environmental risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Given the adverse environmental impacts of the antifoulants currently used in marine antifouling paints, such as copper and booster biocides, it is urgent to identify potential substitutes that are environmentally benign. Here, we examined the degradation of camptothecin (a natural product previously identified as an efficient antifoulant in the laboratory and in the field) under various conditions and evaluated the environmental risks associated with its use as a marine antifoulant. We found that camptothecin was rapidly photolyzed in seawater: the half-life of camptothecin was less than 1 d under a light intensity of 1000–20,000 lx and was approximately 0.17 d under sunlight irradiation. At pH 4 and pH 7, camptothecin had half-lives of 30.13 and 16.90 d, respectively; at 4 °C, 25 °C, and 35 °C, the half-lives of camptothecin were 23.90, 21.66, and 26.65 d, respectively. Camptothecin biodegradation in seawater was negligible. The predicted no-effect concentration (PNEC) of camptothecin was 2.19×10^{-1} μg L^{-1}, while the average predicted environmental concentrations (PECs) in open seas, shipping lanes, commercial harbors, and marinas were 6.14×10^{-7}, 9.39×10^{-7}, 6.80×10^{-3}, and 5.03×10^{-2} μg L^{-1}, respectively. The PEC/PNEC ratio of camptothecin was much lower than 1 (i.e., 2.80×10^{-6}, 4.29×10^{-6}, 3.11×10^{-2}, and 2.30×10^{-1} for open seas, shipping lanes, commercial harbors, and marinas, respectively), indicating that the use of camptothecin as a marine antifoulant posed little environmental risk.

1. Introduction

Marine biofouling, the undesirable settlement and growth of marine fouling organisms on artificial structures (e.g., ship hulls), is a worldwide problem that leads to enormous economic losses and environmental risks (Yebra et al., 2004; Fitridge et al., 2012; Bannister et al., 2019). Marine biofouling can reduce ship speed, increase fuel cost, increase emissions of carbon dioxide and sulfur dioxide, and introduce invasive species (Bressy and Lejars, 2014). Marine paints containing antifoulants are applied to combat biofouling. Metal-based compounds such as tributyltins (TBT) and copper have been widely used as antifoulants. However, these compounds are

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Inhibiting corrosion of aluminum alloy 5083 through *Vibrio* species biofilm

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ABSTRACT

Microbiologically influenced corrosion inhibition (MICI) of aluminum alloy (AA) 5083 by three representative *Vibrio* species were evaluated using electrochemical, surface analysis and surface characterization techniques. Interestingly, all the bacteria exhibited profound inhibitory effect on the corrosion of AA5083 in the chloride-containing culture medium. The MICI mechanism of tested *Vibrio* species is that mature biofilms acted as a diffusion barrier to prevent the penetration of corrosive chloride and consumed up the diffused oxygen by their aerobic respiration. Thus, the biofilm increased the passive range and inhibited the localized attack on the AA5083 surfaces.

1. Introduction

Biofilm, a community of one or more species of bacteria, always forms on the surface of metal and consequently leads to microbiologically influenced corrosion (MIC) [1–3]. MIC on steel, a key structural material in infrastructure, has been extensively explored in the presence of different aerobic and anaerobic bacteria [4–8]. However, studies on non-ferrous materials related MIC are rarely reported. Of those, light-weight aluminum alloys (AA) that are widely used for manufacturing of marine equipment are vulnerable to MIC. In general, AA exhibit a good corrosion resistance in mildly corrosive environment owing to the strong passivity derived from their native oxide layer upon the surface. However, they suffer from localized corrosion attack, in particular, in contact with marine environments [9–11]. In addition, the presence of a great number of microorganisms in seawater degrades the corrosion resistance of AA to some degree given some certain biological metabolism activities [12–14]. Sulfate-reducing bacteria (SRB), always blamed as a major culprit in MIC, caused a severe corrosion of AA5052 [15]. Smirnov et al. [16] also suggested that the main factor responsible for AA destruction by thirteen fungus and six bacterial species was the organic acids secreted in the course of bacterial metabolism.

In contrast, it is recognized that some biological activities of microorganisms contributed positively to the corrosion resistance of metal alloys, termed microbiologically influenced corrosion inhibition (MICI), since the first discovery by Pedersen et al. [17] in 1998. So far, the corrosion inhibitory effect of several bacterial species, such as *Pseudomonas flava, Pseudomonas stutzeri* [18], *Escherichia coli DH5α* [19], and even SRB [20] upon a number of ferrous and non-ferrous alloys have been reported. Different MICI mechanisms, including formation of a barrier film on metal surface [21], oxygen depletion [22], secreting inhibitory enzyme [23], and corrosion inhibition via biomineralization [24] have been proposed. Recently, Moradi et al. [25] have reported an excellent corrosion inhibitory effect of *Vibrio neocaledinocus* sp. which is comparative to that of metallic Ni coating, attracting the attentions to the *Vibrio* species. It is well known that biofilm is responsible for MIC and MICI [26]. *Vibrio* species are Gram-negative, motile with a curved rod shape, the most abundant bacteria in seawater, and they have strong ability to form biofilm on the metal surface. However, there is little known that how *Vibrio* species biofilms influence the corrosion process of the metal matrix where they attached. Thus, it is of great interest to figure out the potential dual roles, MIC or MICI, of different *Vibrio* species with the interactions of AA.

In this work, three representative *Vibrio* species, namely *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Vibrio* sp. EF187016, were selected

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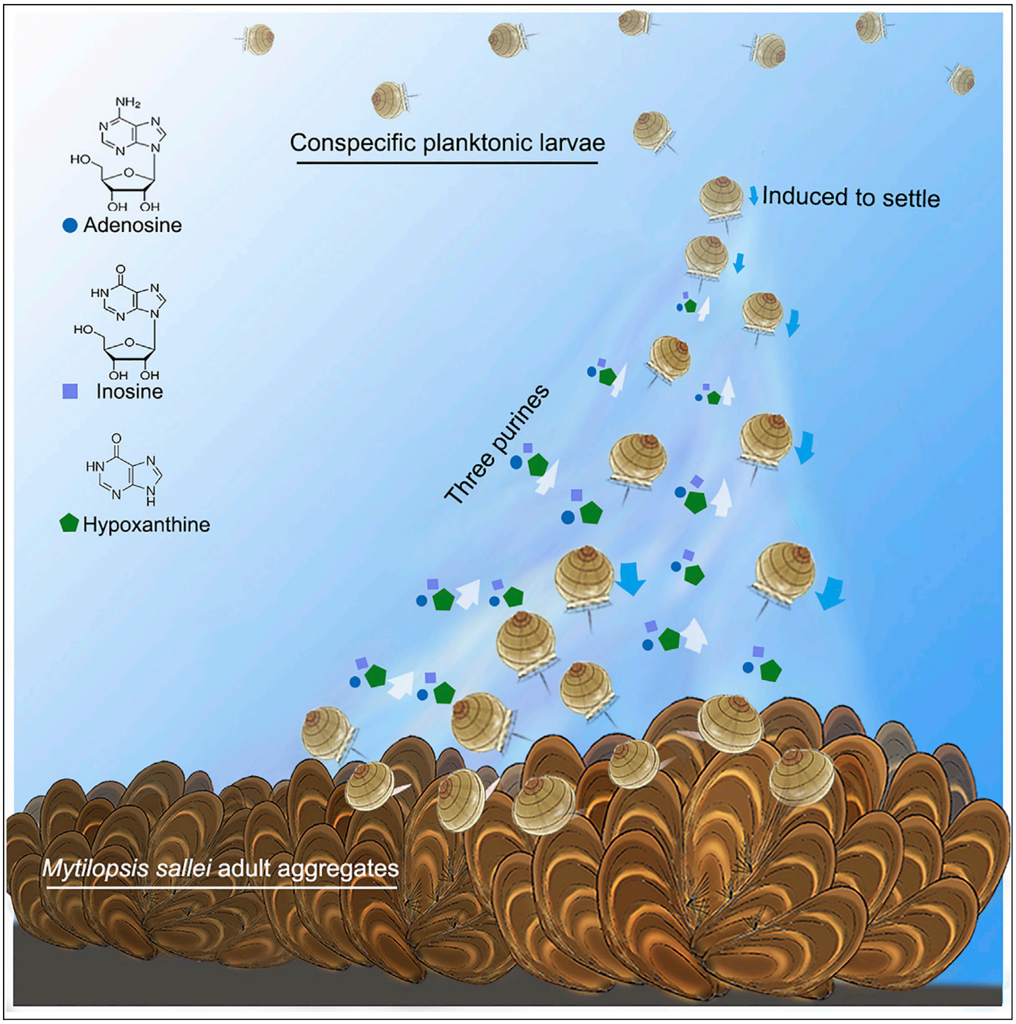
Aggregation Pheromone for an Invasive Mussel Consists of a Precise Combination of Three Common Purines



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HIGHLIGHTS

M. sallei uses a blend of three common simple purines as an aggregation pheromone

The three purines synergistically induce *M. sallei* larvae to settle

Larvae are highly sensitive to the ratio of purines released by conspecific adults

Common metabolites in precise combinations can act as species-specific pheromones

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Aggregation Pheromone for an Invasive Mussel Consists of a Precise Combination of Three Common Purines

Jian He,[1] Qi Dai,[1] Yuxuan Qi,[1] Zhiwen Wu,[1] Qianyun Fang,[1] Pei Su,[1,2] Miaoqin Huang,[1,2] J. Grant Burgess,[3] Caihuan Ke,[1,2,*] and Danqing Feng[1,4,*]

SUMMARY

Most marine benthic invertebrates have a pelagic larval phase, after which they settle preferentially on or near conspecific adults, forming aggregations. Although settlement pheromones from conspecific adults have been implicated as critical drivers of aggregation for more than 30 years, surprisingly few have been unambiguously identified. Here we show that in the invasive dreissenid mussel *Mytilopsis sallei* (an ecological and economic pest), three common purines (adenosine, inosine, and hypoxanthine) released from adults in a synergistic and precise ratio (1:1.125:3.25) serve as an aggregation pheromone by inducing conspecific larval settlement and metamorphosis. Our results demonstrate that simple common metabolites can function as species-specific pheromones when present in precise combinations. This study provides important insights into our understanding of the ecology and communication processes of invasive organisms and indicates that the combination and ratio of purines might be critical for purine-based signaling systems that are fundamental and widespread in nature.

INTRODUCTION

Animal aggregation is one of the most striking behaviors in biology that affects many spatial and temporal processes in ecological systems (Toonen and Pawlik, 1994; Parrish and Edelstein-Keshet, 1999). Despite the cost of increased intraspecific competition, for example, for space, food, and oxygen, aggregation has often been viewed as an evolutionarily advantageous state, in which individuals derive the benefits of protection and reproduction (Danchin and Wagner, 2000; Dzierżyńska-Białończyk et al., 2018). Gregarious settlement is a common phenomenon among marine benthic invertebrates, including mussels, barnacles, oysters, and polychaetes. Most benthic marine invertebrates have a pelagic larval phase, after which they settle preferentially on or near conspecific adults, forming aggregations (Toonen and Pawlik, 1994). The transition from a planktonic to a benthic mode of life is generally accepted as a critical point in their life cycle and is fundamental to understanding population and community dynamics (Shikuma et al., 2014). Although many studies have repeatedly implicated a critical role for pheromones from conspecific adults in the induction of larval settlement forming dense aggregates for more than 30 years, surprisingly few settlement pheromones have been isolated and structurally identified (Burke, 1986; Dreanno et al., 2006). The present understanding of aggregation mechanisms and the evolution of aggregation pheromones is limited.

Invasive dreissenid mussels commonly foul submerged structures with typical high-density aggregations and are well-known ecological and economic pests in aquatic ecosystems (Pimentel et al., 2005; Michalak, 2017). These include the zebra mussel *Dreissena polymorpha*, the quagga mussel *Dreissena rostriformis bugensis* in North America and Europe (Michalak, 2017; Stokstad, 2007), *Mytilopsis leucophaeata* in Europe (Kennedy, 2011), and *Mytilopsis trautwineana* in South America (Aldridge et al., 2008). Dreissenids are dioecious with gametes released directly into the water and fertilized externally (Ram et al., 1996). After a brief free-swimming veliger stage, the pediveliger larvae settle and metamorphose to benthic juveniles, which attach to most substrates with secreted byssal threads leading to fouling. The gregarious settlement of dreissenid mussels causes adverse impacts on aquatic systems and serious cost to industries. The introduction of dreissenid mussels into water pipelines in power plants and water treatment plants causes damage worth billions of dollars in the Great Lakes area (Aldridge et al., 2006). Much research has focused on antifouling compounds in preventing invertebrate settlement (Yebra et al., 2004; Almeida and Vasconcelos, 2015; Qian et al., 2015; Martins et al., 2018). For the control of dreissenid mussels, chlorine has been commonly used in pipelines, but there are environmental concerns about this approach (Meehan et al., 2014). Furthermore, at present no practical

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Articles

Adenosine Triggers Larval Settlement and Metamorphosis in the Mussel *Mytilopsis sallei* through the ADK-AMPK-FoxO Pathway

Jian He, Zhiwen Wu, Liying Chen, Qi Dai, Huanhuan Hao, Pei Su, Caihuan Ke, and Danqing Feng*

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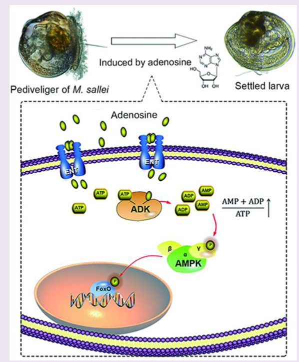
ABSTRACT: Settlement and metamorphosis of planktonic larvae into benthic adults are critical components of a diverse range of marine invertebrate-mediated processes such as the formation of mussel beds and coral reefs, the recruitment of marine shellfisheries, and the initiation of macrobiofouling. Although larval settlement and metamorphosis induced by natural chemical cues is widespread among marine invertebrates, the mechanisms of action remain poorly understood. Here, we identified that the molecular target of adenosine (an inducer of larval settlement and metamorphosis from conspecific adults in the invasive biofouling mussel *Mytilopsis sallei*) is adenosine kinase (ADK). The results of transcriptomic analyses, pharmacological assays, temporal and spatial gene expression analyses, and siRNA interference, suggest that ATP-dependent phosphorylation of adenosine catalyzed by ADK activates the downstream AMPK-FoxO signaling pathway, inducing larval settlement and metamorphosis in *M. sallei*. This study not only reveals the role of the ADK-AMPK-FoxO pathway in larval settlement and metamorphosis of marine invertebrates but it also deepens our understanding of the functions and evolution of adenosine signaling, a process that is widespread in biology and important in medicine.



■ INTRODUCTION

Most marine benthic animals have planktonic larval and benthic juvenile/adult stages. When pelagic larvae are fully developed, they must find an appropriate substrate upon which to settle and metamorphose into juveniles. This transformation is a critical step in the life cycle, as it underpins the propagation and persistence of the population in the marine ecosystem.[1] Larval settlement is central to key processes, such as the formation of mussel beds and coral reefs,[2] the recruitment of marine shellfisheries,[3] and the initiation of macrobiofouling.[4] Furthermore, this planktonic-benthic transition is also important in the study of developmental biology as an early evolutionarily process involving recognition of environmental signals, exploratory behavior on a substratum, and significant changes in morphology and physiology.[1] However, despite the fundamental importance of larval settlement and metamorphosis, our understanding of the underlying molecular mechanisms is limited.

Larval settlement and metamorphosis are triggered by environmental chemical inducers or cues in a wide range of marine benthic animals.[5] However, these cues have only been unambiguously identified in a few cases. These include lumichrome (the metamorphosis inducer for the ascidian *Halocynthia roretzi*) that was isolated from its larval-conditioned seawater,[6] the settlement-inducing protein complex (SIPC) from the barnacle *Balanus amphitrite* purified from conspecific adults,[7] γ-amino butyric acid (GABA), the settlement and metamorphosis inducer for the abalone *Haliotis spp.* identified from abalone mucus,[8] metamorphosis-associated contractile structures (MACs) that induce metamorphosis of the tubeworm *Hydroides elegans* and that were isolated from the bacterium *Pseudoalteromonas luteoviolacea*,[9] and histamine (the settlement and metamorphosis inducer for the sea urchin *Holopneustes purpurascens* isolated from its host alga *Delisea pulchra*).[10]

Invasive dreissenid mussels are well-known invasive species and economic pests in aquatic ecosystems. They can settle gregariously on submerged manmade structures such as water pipelines in power plants, causing serious biofouling problems.[11] Despite their ecological and economic importance, settlement cues for members of the class bivalvia were not characterized until recently, when we demonstrated that the settlement cues for *Mytilopsis sallei* larvae released from conspecific adults consisted of adenosine and its degradation products inosine and hypoxanthine.[12] Adenosine is one of the most important compounds in biochemistry.[13] The adenosine signaling system is ancient in evolution and omnipresent across species and tissues. Adenosine is involved in many essential functions, including immune responses, cell proliferation, differentiation, and programmed cell death during development.[14] Recently, adenosine has also received increasing attention not only as a signaling messenger in internal tissues of organisms but also as a signaling molecule allowing communication between individual

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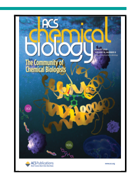

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2-Arachidonoylglycerol as an Endogenous Cue Negatively Regulates Attachment of the Mussel *Perna viridis*

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Endocannabinoids play important roles in the functioning of various physiological systems in humans and non-mammalian animals, including invertebrates. However, information concerning their roles in physiological functions in members of the phylum Mollusca is scarce. Here the hypothesis that the endocannabinoids are involved in mediating settlement of marine invertebrates was tested. Two endocannabinoids [N-arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG)], and two endocannabinoid-like lipids [N-Oleoylethanolamide (OEA) and N-Palmitoylethanolamide (PEA)] were detected in the green mussel *Perna viridis*. In particular, 2-AG was present at significantly higher levels in unattached *P. viridis* compared with attached mussels. The *in vivo* level of 2-AG was inversely correlated with the attachment activity of *P. viridis*. Furthermore, exposure to synthetic 2-AG inhibited attachment of *P. viridis* in a reversible manner. Transcriptomic analysis suggested that up-regulation of 2-AG synthase (Phospholipase C-β, PLC-β) and down-regulation of its degrading enzyme (Monoacylglycerol lipase, MAGL) resulted in higher levels of 2-AG in unattached mussels. A putative mechanism for the negative regulation of mussel attachment by 2-AG is proposed that involves a Ca^{2+}- Nitric oxide (NO)- cyclic guanosine monophosphate (cGMP) pathway. This study broadens our understanding of the evolution and roles of the endocannabinoid system in animals, and reveals an endogenous regulatory cue for mussel attachment.

Keywords: endocannabinoid, 2-AG, mussel, *Perna viridis*, attachment

Abbreviations: 2-AG, 2-Arachidonoyl glycerol; AEA, N-arachidonoyl ethanolamide; AMPAR, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; cGMP, Cyclic guanosine monophosphate; CNRIP1, Cannabinoid receptor-interacting protein 1; DAG, Diacylglycerol; DEG, Differentially expressed genes; DMSO, Dimethyl sulfoxide; FAAH, Fatty acid amide hydrolase; FP1V, Foot protein 1 variant; FPKM, Fragments Per kb per Million reads; FSW, Filtered seawater; $GABA_A$, γ-Aminobutyric acid type A; GPCR, G Protein-Coupled Receptor; GTP, Guanosine-5′-triphosphate; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS, Liquid chromatography–mass spectrometry; MAGL, Monoacylglycerol lipase; MAPK, Mitogen-activated protein kinase; mGluR, Metabotropic glutamate receptor; NAAA, N-Acylethanolamine Acid Amidase; NAPE-PLD, N-acyl phosphatidylethanolamine phospholipase D; NO, Nitric oxide; PEA, N-Palmitoylethanolamide; PKA, Protein kinase A; PKC, Protein kinase C; PLC-β, Phospholipase C β; PPARα, Peroxisome proliferator-activated receptor alpha; S1P, Sphingosine 1-phosphate; THC, Δ^9-tetrahydrocannabinol; TRPV1, Transient receptor potential cation channel, subfamily V, member 1; VGCC, Voltage-gated calcium channels.

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The effects of model polysiloxane and fouling-release coatings on embryonic development of a sea urchin (*Arbacia punctulata*) and a fish (*Oryzias latipes*)

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ABSTRACT

In recent decades attention has focused on the development of non-toxic fouling-release coatings based on silicone polymers as an alternative to toxic antifouling coatings. As fouling-release coatings gain market share, they will contribute to environmental contamination by silicones. We report effects of eight model polysiloxane and three commercial foul-release coatings on embryonic development of sea urchins and fish, Japanese medaka. We used model coatings because they have known composition and commercially available components and molecules leaching from these coatings have been partially characterized. The commercial fouling-release coatings are purported to be non-toxic and components are proprietary. Our goal was to expose embryos of well studied model animals to the coatings to determine if the complex mixtures leaching from the coatings impact development. Urchins were chosen because development is rapid and embryos can enter the non-slip layer over surfaces. Medaka was chosen because the female deposits the sticky eggs onto the anal fin and then scrapes them off onto surfaces. Embryos were confined in water over coatings in 24 well plates. Fresh model coatings had no effect on urchin development while commercial fouling-release coatings inhibited development. Fish embryos had delayed hatching, increased mortality of hatchlings and dramatically decreased ability of hatchlings to inflate the swim bladder and reduced hatching success on all coatings. After one-month immersion of coatings in running seawater to simulate initial application in the marine environment, sea urchin embryos died when placed over model silicones. Effects of the commercial coatings were reduced but included retarded development. Effects on fish embryos over leached coating were reduced compared to those of fresh coating and included decreased hatching success, decreased hatchling survival and inability to inflate the swim bladder for commercial coatings. These findings suggest, similar to medical conclusions, compounds leaching from silicone coatings can impact development and the topic deserves study.

1. Introduction

Negative environmental impacts of biocides such as tributyltin and copper have led to regulations and bans on their use in marine biofouling control coatings (Rittschof, 2001; van Wezel and van Wlaardingen, 2004; Yebra et al., 2004). A result is research and development efforts focused on non-toxic measures such as fouling-release coatings. These coatings present a surface which reduces the adhesion strength of settling organisms (Berglin et al., 2001; Clare, 1998) enabling cleaning by pressurized water jets or in some cases by hydrodynamic forces generated as the ship moves (Berglin et al., 2003). Surface properties of the coatings including surface free energy, elastic modulus and thickness are important to the fouling-release performance (Brady and Singer, 2000; Webster and Chisholm, 2010).

The concept of fouling-release coatings has been in development for over three decades (Rittschof, 2009). A great deal of research has been conducted on fouling-release coating systems based upon silicones and fluoropolymers. Silicone polymers, based on poly-dimethyl-siloxane (PDMS), appear to out-perform fluoropolymers and be a viable fouling-release coating system (Rittschof, 2009; Stein et al., 2003). Incorporation of low molecular weight silicone oils into silicone coatings improves their fouling-release properties (Meyer et al., 2006; Milne, 1977; Rittschof et al., 2008; Stein et al., 2003; Truby et al., 2000). The composition of commercial coatings is proprietary.

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Sea-trial research on natural product-based antifouling paint applied to different underwater sensor housing materials

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ABSTRACT

Biofouling is a common challenge for underwater sensors, especially for long-term *in situ* monitoring in marine environments. In this study, we assessed the antifouling efficacy of a paint containing a natural product camptothecin (CPT) on six materials (316 L stainless steel, TC4 titanium alloy, 7075 aluminum alloy, polyoxymethylene, polyvinyl chloride, and Teflon), which are frequently used in the construction of underwater sensor housings. Additionally, a buoy-based sea-trial was performed to test the antifouling performance of the CPT-based paint on housings of three *in situ* sensors used for practical seawater monitoring applications, namely a spectrophotometer for chemical oxygen demand (COD) measurements and two fluorimeters for biochemical oxygen demand (BOD) and chlorophyll *a* (Chl *a*) concentration measurements. The results showed significantly lower macrofouling coverage on the areas painted with the CPT-based paint compared to the unpainted areas for each tested material over 9 months of seawater immersion. The CPT-based paint exhibited different antifouling performance for the different materials; in particular, it exhibited better antifouling performance on the plastic materials compared to the metal materials. Furthermore, when applied on submersible sensor housings in the sea-trial test, the CPT-based paint kept the housings of the COD sensor and the Chl *a* sensor clean for over 4 months. In addition, the paint prevented fouling of the BOD sensor housing even after 6 months of seawater immersion. Thus, our results suggest that the CPT-based paint could be used as a potential solution to control the biofouling of sensor housings for long-term *in situ* applications in marine environments.

1. Introduction

Submersible instruments are important for *in situ* ocean observations, marine investigations, scientific research, seawater quality monitoring, and emergency management, including a large number of chemical, acoustic, electrical, optical, and biological sensors. For instruments deployed underwater, biofouling can be a serious issue that affects their operation, maintenance, and data integrity (Delgado et al., 2021). In addition, biofouling on *in situ* sensor surfaces can shorten their operating lifetime, increase the cost and frequency of maintenance, and result in signal drift and data errors (Whelan. and Regan., 2006). This is especially true for long-term *in situ* monitoring sensors, and biofouling has been considered as a key limiting factor that affects deployment duration. Therefore, an effective method to control fouling is needed for applications that rely on *in situ* sensors in marine environments.

Several antifouling strategies have been proposed to protect the sensing surfaces of sensors (e.g., optical windows and filtration membranes), including the use of wipers, brushes, copper shutters, ultraviolet (UV) light irradiation, and ultrasonic treatment (Whelan. and Regan., 2006; Delauney et al., 2010; Delgado et al., 2021). As an important

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Conspecific cues that induce spore settlement in the biofouling and green tide-forming alga *Ulva tepida* provide a potential aggregation mechanism

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A B S T R A C T

Ulva, an important biofouling and green tide-forming alga, forms aggregations that have adverse economic and ecological impacts. However, little is known regarding the aggregation mechanism of *Ulva*. Knowledge of *Ulva* aggregation provides clues for controlling this alga in marine biofouling and green tides. We hypothesized *Ulva* contains conspecific settlement cue(s). Hexane and dichloromethane extracts of *U. tepida* fronds were found to induce the settlement of conspecific spores. From these extracts, two bioactive compounds that induce spore settlement in *U. tepida* were isolated: C16:4 at 5 μg mL^{-1} and di-n-butyl phthalate at 0.1–10 μg mL^{-1}. Fatty acids with different carbon chains (C_{16}–C_{18}) and degrees of saturation, C16:1 and C18:2, previously reported to be present in *U. tepida* fronds, showed significant inducing activity for spore settlement. C16:1, C16:4, C18:2, and di-n-butyl phthalate all triggered dense settlements of *U. tepida* spores. The results support our hypothesis and provide insight into possible aggregation mechanisms for *Ulva*.

1. Introduction

Ulva (= *Enteromorpha*) spp. are cosmopolitan intertidal green macroalgae (Kirkendale et al., 2013; Wichard et al., 2015) and are the most common macroalgal biofoulers in the world (Callow, 1986; Hoipkemeier-Wilson et al., 2004). The production of motile spores and settlement of the spores on substrata are key stages in the *Ulva* life cycle. Habitat selection occurs during the progression from planktonic spores to benthic sporelings. Spores actively swim, explore, and sense potential surfaces (Reed et al., 1992; Pickett-Heaps et al., 2010; Heydt et al., 2012), following which they attach to a surface and develop into new plants.

In the field, *Ulva* settles in aggregates (Fig. 1) often on manmade surfaces, causing adverse economic and ecological impacts. Biofouling results in increased fuel consumption and more costly dry-docking for ships (Abbot et al., 2000; Callow and Callow, 2002) as well as other substantial damage to marine artificial structures (Messano et al., 2009; Venkatesan et al., 2017). Green tides are another example of *Ulva* aggregation (Teichberg et al., 2010; Bast et al., 2014; Hu et al., 2014; Gao et al., 2017, 2018). Green tides are large volumes of algal biomass on beaches and in coastal waters. Green tides harm tourism-based economies, interrupt traditional fisheries and impact aquaculture (Ye et al., 2011; Smetacek and Zingone, 2013). However, in the current literature, little is known regarding the aggregation mechanism of *Ulva*.

Transition from a planktonic propagule to a benthic mode of life is common in sessile fouling organisms. Gregarious settlement, aggregation, is also very common. The biological advantages of living in dense groups include enhanced cross-fertilization success of propagules, decreased probability of predation (Highsmith, 1982), and reduced turbulence from wave action (Railkin, 2004). Many studies demonstrate chemical cues associated with conspecific juveniles and adults of invertebrates induce the settlement of planktonic larvae on or near adults, resulting in aggregation. The original work on gregarious settlement resulting in aggregation dates to the 1950s (Barnes, 1953; Crisp and Knight-Jones, 1953; Hidu, 1969). The settlement of Pacific oyster (*Crassostrea gigas*) larvae is guided by glycoproteins contained in the shell of conspecifics (Vasquez et al., 2013). The larvae of the barnacle *Balanus* (= *Amphibalanus*) *amphitrite* are induced to settle by water-

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Effective natural antifouling compounds from the plant *Nerium oleander* and testing

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ABSTRACT

One major challenge to commercialization of natural antifoulants is to find the effective antifouling substances from natural flora and fauna with sufficient amount available. In this study, four cardenolides, odoroside A, digitoxigenin, oleandrin and odoroside H, were isolated from a widely distributed plant *Nerium oleander* L. These four compounds and their eight analogues were then evaluated for antifouling activity against the barnacle *Balanus albicostatus* cyprids. All of the tested compounds showed a strong inhibitory activity against barnacle settlement, with EC_{50} values ranging from 0.58 to 230.67 ng ml^{-1}. Additionally, evaluation of their lethality against a non-target organism *Artemia salina* L., revealed LC_{50} values of 17.23 to above 100 μg ml^{-1}, indicating moderate to low toxicity towards *A. salina*. Furthermore, investigation of the field antifouling performance of three *N. oleander* extracts containing cardenolides by incorporation into coatings revealed significant antifouling efficiency in marine water for 30 days. These findings indicate the commercial potential for these natural antifouling products from *N. oleander* as natural antifoulants.

1. Introduction

Marine biofouling poses serious global economic problems (e.g., reduced ship speed and increased fuel consumption) and environmental risks (e.g., increased emission of greenhouse gases and dissemination of invasive foreign species) (Fletcher, 1988; Schultz, 2007; Hellio, 2010; Poloczanska and Butler, 2010; Maréchal and Hellio, 2009; Chan et al., 2014). Antifouling biocides such as organotin, copper oxide and some herbicides have been widely applied to control marine biofouling. However, there are currently bans and regulations on the use of these antifoulants due to their negative environmental impacts (Burgess et al., 2003; Bellas, 2006; Callow and Willingham, 1996; Thomas and Brooks, 2010). Hence, there is an urgent need for environmentally friendly antifouling agents. As a promising source of such alternatives, natural antifouling products have received a lot of attention.

Studies of natural antifouling products have focused on isolating antifouling active secondary metabolites from marine organisms, including marine bacteria, fungi, algae, sponges, corals, bryozoans and ascidians (Clare, 1996; Omae, 2003; Qian et al., 2009; Chen et al., 2013; Almeida and Vasconcelos, 2015). Many marine natural products with antifouling activity have been found and identified as terpenoids, steroids, saponins, alkaloids, fatty acids, amino acids, polyketides and polyphenolics (Fusetani, 2004, 2011; Omae, 2006; Qian et al., 2015). However, these are usually difficult to produce on a large-scale for commercial because they are generally not available in sufficient quantities from marine organisms and difficult to chemically synthesize at a low cost (Raveendran and Mol, 2009; Feng et al., 2009a; Peérez et al., 2014b; Qian et al., 2015). When compared with marine organisms, many terrestrial plants can be easily harvested on a commercial scale because of their wide distribution and/or mass cultivation (Feng et al., 2009b; Pérez et al., 2014a). Moreover, a few studies have confirmed the antifouling activity of extracts and compounds from terrestrial plants. *Trans*-6-, 8- and 10-shogaols, isolated from the roots of the ginger *Zingiber officinale* Roscoe, inhibited attachment of the blue mussel *Mytilus edulis galloprovincialis*, while *trans*-8-shogaol showed antifouling activity in the field (Etoh et al., 2002). Bioassay-guided isolation of acetone extract from the stem of the betel *Piper betle* led to the discovery of four piperamides with antifouling activity, and one of their synthesized analogues, 1-[1-oxo-7-(3′,4′-methylenedioxyphenyl)-6E-heptenyl]- piperidine, exhibited inhibitory activity against barnacle

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Short communication

Antifouling activity against bryozoan and barnacle by cembrane diterpenes from the soft coral *Sinularia flexibilis*

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A B S T R A C T

In the present study, seven cembrane diterpenes were isolated from the soft coral *Sinularia flexibilis* by Toyopearl HW-40 column chromatography and High Performance Liquid Chromatography (HPLC). The diterpenes were identified as epoxycembrane A (**1**), sinularin (**2**), sinulariolide (**3**), (1*R*,13*S*,12*S*,9*S*,8*R*,5*S*,4*R*)-9-acetoxy-5,8:12,13-diepoxycembr-15(17)-en-16,4-olide (**4**), 11-dehydrosinulariolide (**5**), (−)14-deoxycrassin (**6**) and dihydrosinularin (**7**). The antifouling activity of these compounds was examined by settlement assays, using the larvae of the bryozoan *Bugula neritina* and the barnacle *Balanus albicostatus*. With the exception of compound **2**, all compounds indicated significant antifouling activity and a variety of EC_{50} values. In particular, compound **6** exhibited remarkable anti-settlement activity against the two biofoulers (EC_{50} for *B. neritina* 3.90 μg ml^{-1}; EC_{50} for *B. albicostatus* 21.26 μg ml^{-1}) as well as low toxicity against *B. albicostatus* larvae ($LC_{50} > 100$ μg ml^{-1}), suggesting its potential as an environmentally friendly antifoulant. This is the first report on the antifouling activity of compounds **1** and **4**–**7**, further demonstrating the involvement of cembrane diterpenes in the chemical defense of soft corals against surface fouling.

1. Introduction

Toxic antifoulants such as tributyltin and copper have been widely used on the surfaces of artificial structures submerged in the sea in order to deter marine fouling organisms (Yebra et al., 2004; Muynck et al., 2009; Dafforn et al., 2011). However, a growing awareness of their adverse environmental impacts has led to strict regulations on their use as marine antifoulants (Thomas and Brooks, 2010; Dafforn et al., 2011). This has triggered the search for environmentally friendly natural antifouling products. A great deal of effort has been directed towards the screening of natural product antifoulants (NPAs) from marine organisms, especially sessile, soft-bodied marine species such as macroalgae, sponges and soft corals (Fusetani, 2011; LimnaMol et al., 2011; Patro et al., 2012; Qian et al., 2015). Despite being exposed to surface biofouling in the marine environment, some of these species are able to remain free of fouling. As they lack the necessary behavioral or physical defenses, it is believed that they resist epibiosis via the production of antifouling secondary metabolites (Schmitt et al., 1998; Dobretsov et al., 2004, 2015). Numerous NPAs have been isolated from sessile, soft-bodied marine organisms, suggesting that they are promising sources of NPAs.

Soft corals are one of the most prolific sources of bioactive marine natural products. A variety of secondary metabolites have been extracted from soft corals, indicating various cytotoxic, antibacterial, anti-inflammatory, antiviral and antifouling bioactivities (Wen et al., 2008; Lai et al., 2013;Cheng et al., 2015; Gomaa et al., 2015; Taira et al., 2015). Soft corals of the genus *Sinularia* are particularly well-known for containing bioactive substances with interesting chemical structures, including diterpenes, sesquiterpenes, polyhydroxylated steroids and polyamine compounds (Kamel and Slattery, 2005; Chen et al., 2012).

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Antifouling activities of hymenialdisine and debromohymenialdisine from the sponge *Axinella* sp.

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A B S T R A C T

Being physically unprotected sessile organisms, marine sponges are thought to protect themselves from surface fouling through the use of antifouling secondary metabolites. In this study, the sponge *Axinella* sp. was extracted with methanol and then partitioned between organic solvents and water. Two main compounds, hymenialdisine (HD) and debromohymenialdisine (DBH), were isolated from the n-BuOH layer using Sephadex LH-20 and C-18 column chromatography. The antifouling activity of HD and DBH were evaluated using the test of byssus thread production with the green mussel *Perna viridis*, and the settlement assays with the bryozoan *Bugula neritina* larvae and the green alga *Ulva prolifera* spores. Both HD and DBH were found to exhibit significant antifouling activities against *P. viridis* (EC_{50} values of 31.77 and 138.18 μg ml^{-1}, respectively), *B. neritina* (EC_{50} values of 3.43 and 8.17 μg ml^{-1}, respectively) and *U. prolifera* (EC_{50} values of 8.31 and 0.67 μg ml^{-1}, respectively). Our results suggested that HD and DBH may play a role in chemical defense against fouling in *Axinella* sp.

1. Introduction

There have been numerous studies reporting the presence of biologically active secondary metabolites in marine organisms (e.g. Faulkner, 1998, 2000, 2001, 2002; Tziveleka et al., 2003; Blunt et al., 2009). Sponges (Porifera) in particular, are the most prolific source of marine natural products, and have long been in focus of natural product chemists (Faulkner, 1998, 2000, 2001, 2002; Sipkema et al., 2005). The chemical diversity of compounds isolated from sponges is remarkable. Compounds include sterols, terpenoids, alkaloids, fatty acids, macrolides, saponins, nucleosides, peroxides, and amino acid derivatives (Faulkner, 1998; Sipkema et al., 2005). These compounds, proven to be antitumor, antiviral, antiinflammatory, antimalarial, immunosuppressive, or antibiotic, exhibited significant potential in drug development (Munro et al., 1999; Sipkema et al., 2005; Mayer et al., 2009). Although a great deal of research has been done on chemical analyses and pharmacological activities of natural products from sponges, the ecological functions of most of these bioactive compounds in sponges remain to be determined.

Being sessile and soft-bodied organisms, marine sponges are physically vulnerable to predation, competition for space, attacks from potential pathogens, and surface fouling. It is commonly accepted that sponges protect themselves using powerful chemical defenses through the production of bioactive metabolites (Proksch, 1994; Engel and Pawlik, 2000; Laport et al., 2009). For example, presence of natural products with antifouling activity have been reported in many sponges and suggested to be involved in the defense of sponges against fouling organisms (Willemsen, 1994; Tsoukatou et al., 2002; Hellio et al., 2005; Limna Mol et al., 2009).

The sponge *Axinella* sp. was observed to be free of epibiosis in the field, suggesting that this species may possess certain antifouling mechanisms. Our preliminary study found that the crude extract from *Axinella* sp. exhibited antifouling activity. Two main alkaloids, hymenialdisine (HD) and debromohymenialdisine (DBH), were isolated from the *Axinella* sp. crude extract using a bioassay-guided approach. It is noteworthy that HD and DBH have been reported to exhibit inhibitory effects against a number of protein kinases in pharmacological studies and have shown their

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Integration of PEGylated Polyaniline Nanocoatings with Multiple Plastic Substrates Generates Comparable Antifouling Performance

Chen-Xi Shuai, Yuan He, Pei Su, Qiaoling Huang, Deng Pan, Qingchi Xu,* Danqing Feng,* and Yuan Jiang*

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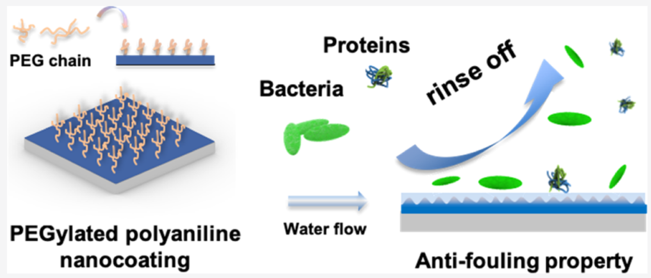
ABSTRACT: Conducting polymer nanocoatings render plastics to possess interesting optical, chemical, and electrical properties. It nevertheless remains technically challenging to deposit uniform conducting polymer nanocoatings on ambient plastic substrates ascribed to the inert and varied chemical properties of plastics and the notorious processability of conducting polymers. Previous studies have made progress in delivering various conducting polymer thin films *via* oxidative chemical vapor deposition. Herein, we develop a solution-based approach to polyaniline (PANI) and PEGylated PANI nanocoatings on multiple engineering plastics followed by evaluating their antifouling performance. The procedure relies on the formation of uniform, lyotropic $V_2O_5 \cdot nH_2O$ thin films on plastics assisted by a surfactant—sodium *N*-lauroylsarcosinate. Next, *in situ*, oxidative polymerization causes the formation of nanofibrous PANI nanocoatings. Finally, interfacial functionalization leads to PEGylated PANI nanocoatings, and the steric nanolayer effectively repels the adsorption of bovine serum albumin and the attachment of the bacterium *Pseudoalteromonas* sp. on the surface. It is worth noting that the antifouling properties rely mainly on the presence of PEGylated PANI nanocoatings, irrespective of the type of plastic substrates underneath. The current study therefore opens an avenue for the solution-based delivery of conducting polymer-based, functional nanocoatings on hydrophobic substrates in a controllable manner with the availability of further modification.



■ INTRODUCTION

Plastics are increasingly important materials used in the fabrication of biosensors, biomedical and marine equipment, food storage containers, water purification membranes, for example.[1−7] Conducting polymers exhibit intrinsic electric conductivity, tunable hydrophilicity, availability of chemical modification, chemical and mechanical stability, and biocompatibility, and their coupling with plastics can pave way for the fabrication of high-performance devices with the availability of the integration of emerging functions.[8] The prevalent processing method disperses micro- and nano-sized particles of conducting polymers in plastic matrices for the fabrication of functional composites. Nevertheless, high doping levels will inevitably cause a dramatic change of the intrinsic properties of the plastics and the deterioration of their long-termed durability.

As an alternative approach, conducing polymer-based films render plastics to possess interesting optical, chemical, and electrical properties, and in the meantime, can maintain the bulk properties of the plastics.[9] It nonetheless remains technically challenging to design general approaches to deposit uniform conducting polymer thin films on plastic substrates ascribed to the poor processability of conducting polymers. Traditional, solution-processed deposition of conducting polymers on plastic substrates often led to thick coatings usually hundreds of nanometers in thickness or beyond, and poor adhesion is inevitable owing to the stiffness of conducting polymer chains and their limited van der Waals interactions with the substrate.[10,11] The solution-based method only made success in delivering poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) thin films on plastic substrates owing to the good solubility of PEDOT in water and organic solvents in the presence of PSS dopants.[12,13] The increased hydrophilicity in the resulting PEDOT:PSS coatings nevertheless brings in long-term stability issues owing to the dissociation of the PSS constituents. In recent years, the Gleason group proposed an emerging method, namely, oxidative chemical vapor deposition to synthesize conducting polymer thin films.[14,15] This green approach is advantageous for providing precise thickness control and good adhesion of thin films on substrates, processing simplicity, and importantly,

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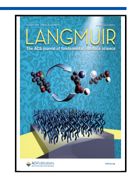

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Strong adhesion of poly(vinyl alcohol)–glycerol hydrogels onto metal substrates for marine antifouling applications†

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Hydrogels can be used as an alternative coating material for ships against marine biofouling. However, the adhesion of wet and soft hydrogels onto solid metals remains a challenging problem. Here we report the adhesion of a typical hydrogel material, poly(vinyl alcohol) (PVA)–glycerol hydrogel, onto stainless steel substrates and the antifouling potency of the adhered PVA–glycerol hydrogels. Poly(allylamine hydrochloride) (PAH) hydrogel and ethyl α-cyanoacrylate (ECA) are used as the binders, and they are found to be able to firmly bond the PVA–glycerol hydrogels onto the stainless steel substrates. The PAH hydrogel does not affect the mechanical properties of the PVA–glycerol hydrogel during use, but it tends to lose the adhesive ability in a dehydrating environment. In contrast, the ECA adhesive can maintain strong bonding between PVA–glycerol hydrogels and substrates upon several water losing/water absorbing cycles, despite some negative effects on the strength of the PVA–glycerol hydrogel. Biological experiments show that the PVA–glycerol hydrogel has a strong settlement-inhibiting effect on the barnacle *Balanus albicostatus*, suggesting that combining the PVA–glycerol hydrogel with ECA adhesive may have promising applications in marine antifouling.

1 Introduction

Marine biofouling, the accumulation of marine fouling organisms on submerged surfaces,[1] has been a serious problem for the development of marine economy, since these attached organisms can slow down the vessels and cause extra fuel consumption up to 40%, leading to billions of dollars in waste.[2,3] The fouling organisms can also corrode the surface they are in contact with, resulting in a shortened life of the hulls.[4,5] This problem used to be solved by tin-containing self-polishing coatings since organotin compounds can kill marine organisms efficiently. But the global prohibition of coating with tributyltin (TBT) in 2008, due to its negative effects on marine environments, raised this issue again.[6] Though copper/zinc[7,8] and organic biocides[9–11] are used as substitutes, copper related coatings are also toxic and the toxicity of organic biocides towards marine environments is still under investigation.[12–17] Therefore, environment friendly coatings are urgently needed in the current market. Compared with killing the biofouling organism, preventing or reducing settlement of the fouler on submerged surfaces is more favorable for environment protection.[15] Thus, more researchers are willing to focus on green coatings like fouling release, fouling resistant and non-leaching biocide coatings.[1,18,19] Several green coatings such as PEG-based coatings,[20–22] and PDMS-based coatings[23–25] have been reported in recent years.

Among the diverse coating materials, hydrogels, which are soft and wet materials with 3D networks, are considered to be promising candidates with high antifouling performance against marine organisms. The super hydrophilic characteristic of hydrogel materials makes them absorb a large amount of water into the 3D networks and form a highly hydrated layer on their surface which can prevent the adhesion of proteins or microorganisms. Besides, the swollen hydrogels possess a soft and highly elastic nature while most marine organisms prefer to attach to hard surfaces. Murosaki *et al.*[26,27] investigated the antifouling behaviors of a series of synthetic hydrogels against barnacles both in the laboratory and in a long-term marine environment experiment, and the results actually evidenced the efficient antifouling performance of the hydrogels. However, there is an emerging issue for practical applications regarding how to coat hydrogels onto the required substrates with steady adhesion. Xie *et al.*[28] and Hong *et al.*[29] reported a polymeric coating made of cross-linked poly(methyl methyacrylate-*co*-tributylsilyl

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ORIGINAL ARTICLE

The Plant Alkaloid Camptothecin as a Novel Antifouling Compound for Marine Paints: Laboratory Bioassays and Field Trials

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Abstract

The extensive use of copper and booster biocides in antifouling (AF) paints has raised environmental concerns and the need to develop new AF agents. In the present study, 18 alkaloids derived from terrestrial plants were initially evaluated for AF activity using laboratory bioassays with the bryozoan *Bugula neritina* and the barnacle *Balanus albicostatus*. The results showed that 4 of the 18 alkaloids were effective in inhibiting larval settlement of *B. neritina*, with an EC_{50} range of 6.18 to 43.11 μM, and 15 of the 18 alkaloids inhibited larval settlement of *B. albicostatus*, with EC_{50} values ranging from 1.18 to 67.58 μM. Field trials that incorporated five alkaloids respectively into paints with 20% *w*/*w* indicated an in situ AF efficiency of evodiamine, strychnine, camptothecin (CPT), and cepharanthine, with the most potent compound being CPT, which also exhibited stronger AF efficiency than the commercial antifoulants cuprous oxide and zinc pyrithione in the field over a period of 12 months. Further field trials with different CPT concentrations (0.1 to 20% *w*/*w*) in the paints suggested a concentration-dependent AF performance in the natural environment, and the effective concentrations to significantly inhibit settlement of biofoulers in the field were ≥ 0.5% *w*/*w* (the efficiency of 0.5% *w*/*w* lasted for 2 months). Moreover, CPT toxicity against the crustacean *Artemia salina*, the planktonic microalgae *Phaeodactylum tricornutum* and *Isochrysis galbana*, was examined. The results showed that 24 h LC_{50} of CPT against *A. salina* was 20.75 μM, and 96 h EC_{50} (growth inhibition) values of CPT to *P. tricornutum* and *I. galbana* were 55.81 and 6.29 μM, respectively, indicating that CPT was comparatively less toxic than several commercial antifoulants previously reported. Our results suggest the novel potential application of CPT as an antifoulant.

Keywords Antifouling compound · Camptothecin · Alkaloid · Biofouling · Natural antifoulant

Introduction

Biofouling control is essential for maritime industries because settlement of marine fouling organisms on surfaces of artificial submerged structures causes serious economic problems (Yebra et al. 2004). Particularly for ships, fouling of hulls increases frictional drag, reduces ship speed, and leads to higher fuel cost (Townsin 2003). The widely used technology to control marine biofouling is antifouling (AF) paint that contains biocides. Organotin-based paints are highly effective, but have been banned due to their highly toxic effects on non-target organisms (IMO 2008; Yebra et al. 2004). Currently, AF paints containing copper and organic booster biocides (e.g., diuron, Irgarol 1051, Sea-Nine 211, dichlofluanid, and chlorothalonil) are widely applied as alternative. However, these antifoulants can also pose adverse impacts on the marine environment, which subsequently led to bans and regulations in various countries (Price and Readman 2013; Thomas and Brooks 2010). Consequently, increasing environmental concern and regulatory pressure has prompted the development of novel non- or less toxic AF agents.

Natural product antifoulants (NPAs) are considered as promising sources of environmentally friendly antifoulants (Qian et al. 2015). Although numerous compounds have been found to be AF active, there are obstacles for the commercial use of NPAs in marine antifouling paints. One major obstacle is the supply of NPAs. Most NPAs reported are derived from marine macroorganisms (including macroalgae, sponges, soft

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ORIGINAL ARTICLE

Pyrethroids as Promising Marine Antifoulants: Laboratory and Field Studies

Danqing Feng · Caihuan Ke · Shaojing Li · Changyi Lu · Feng Guo

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Abstract Due to the regulations and bans regarding the use of traditional toxic chemicals against marine fouling organisms and the practical impediments to the commercialization of natural product antifoulants, there is an urgent need for compounds that are antifouling-active, environmentally friendly, and have a potential for commercial application. In this study, a series of common, commercially available pyrethroid products, which are generally used as environmentally safe insecticides, was evaluated for antifouling activity in the laboratory using an anti-settlement test with cyprids of the barnacle *Balanus albicostatus* and also in a field experiment. Laboratory assay showed that all eleven pyrethroids (namely, rich *d-trans*-allethrin, Es-biothrin, rich *d*-prallethrin, S-prallethrin, tetramethrin, rich *d*-tetramethrin, phenothrin, cyphenothrin, permethrin, cypermethrin, and high active cypermethrin) were able to inhibit barnacle settlement (EC_{50} range of 0.0316 to 87.00 μg/ml) without significant toxicity. Analysis of structure–activity relationships suggested that the cyano group at the α-carbon position had a significant influence on the expression of antifouling activity in pyrethroids. In the field, the antifouling activity of pyrethroids was further confirmed, with the most potent pyrethroids being cypermethrin and high active cypermethrin, which displayed efficiency comparable with that of tributyltin. In summary, our investigation indicated that these pyrethroids have a great and practical commercial potential as antifouling agents.

Keywords Pyrethroids · Marine antifoulants · Commercialization · Antifouling activity · Barnacle · Structure–activity relationships

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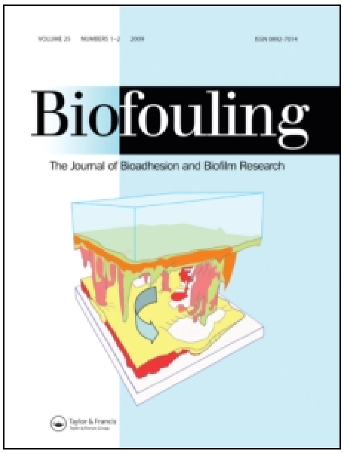
Introduction

It is well known that marine fouling organisms, settling on ship hulls and other man-made constructions immersed in the sea, constitute a worldwide technical and economical problem (Yebra et al. 2004; Townsin 2003; Rittschof 2000). The most widespread solution to fouling is based upon coatings containing toxic chemicals such as organotin and copper (Alberte et al. 1992; Yebra et al. 2004). However, these compounds have been found to be highly toxic to non-target species, to persist in the environment, and to pollute the marine ecosystem (Ellis 1991; Cardwell et al. 1999; Clare et al. 1992). This has resulted in regulations and bans on their use in many countries (Rittschof 2001; van Wezel and van Wlaardingen 2004). In the wake of this, a great deal of research has been focused on finding new antifouling agents which are environmentally friendly.

In many studies, it is suggested that marine natural products with antifouling activity are promising and eco-friendly alternatives for classical antifoulants, since these natural products already exist in the marine environment and are biodegradable (e.g., Abarzua and Jakubowski 1995; Clare 1996, 1998; Burgess et al. 2003; Fusetani 2004; Hellio et al. 2005). So far, more than 100 marine natural product antifoulants have been isolated and identified, including mainly terpenoids, steroids, fatty acids, amino acids, heterocyclics, acetogenins, alkaloids, and polyphenolics (Targett 1997; Rittschof 1999, 2001; Yebra et al.



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Herbal plants as a promising source of natural antifoulants: evidence from barnacle settlement inhibition

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Antifouling activity of terpenoids from the corals *Sinularia flexibilis* and *Muricella* sp. against the bryozoan *Bugula neritina*

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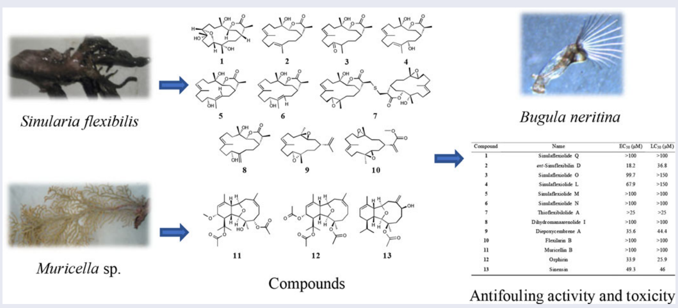
ABSTRACT
Marine natural products are promising sources of green antifoulants. Here, a new compound (**1**) was isolated from the soft coral *Sinularia flexibilis*. This compound, another nine cembranoids (**2**–**10**) from *S. flexibilis*, and three eunicellin-type diterpenoids (**11**–**13**) from the gorgonian *Muricella* sp. were tested for antifouling activity against larval settlement of the bryozoan *Bugula neritina*. Compounds **2**, **3**, **4**, **9**, **12**, and **13** exhibited significant antifouling activity, with EC_{50} values of 18.2, 99.7, 67.9, 35.6, 33.9, and 49.3 μM, respectively. Analysis of the structure-activity relationships suggested that the hydroxy group at C-13 in compound **4** reduced its antifouling activity.



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Antifouling terpenoids; *Sinularia flexibilis*; *Muricella* sp.; *Bugula neritina*; structure-activity relationship (SAR)

1. Introduction

Marine biofouling on ships and other submerged structures is a persistent problem that causes serious economic losses globally [1]. Metal-based antifoulants such as copper and tributyltin have been widely used to prevent biofouling [1]. Owing to the negative environmental effects of toxic metal-based antifoulants [1], environmentally

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Insights into the planktonic to sessile transition in a marine biofilm-forming *Pseudoalteromonas* isolate using comparative proteomic analysis

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ABSTRACT: Bacterial biofilms play an important role in marine biofouling. The formation of a biofilm starts when marine bacterial cells transition from a planktonic to an attached state. However, the molecular mechanisms involved in this transition are poorly understood. Here, 51 strains of marine bacteria were isolated from natural biofilms growing on submerged artificial surfaces (glass slides, epoxy panels, and bridge pillars) and evaluated for their biofilm-forming capacity. Eleven strains formed relatively strong biofilms and 16S rRNA gene sequence analysis indicated that they belonged to the genera *Leisingera*, *Roseobacter*, *Pseudoalteromonas*, *Alteromonas*, *Tenacibaculum*, *Vibrio*, *Chryseobacterium*, *Aquimarina*, and *Acinetobacter*. Strain *Pseudoalteromonas* sp. W-7 showed efficient and rapid attachment and was therefore chosen for further study. An iTRAQ-based comparative proteomic analysis of planktonic and attached strain W-7 cells was carried out. A total of 3468 proteins were identified, of which 163 showed significant differential expression (120 down-regulated and 43 up-regulated in attached cells relative to planktonic cells). KEGG (Kyoto encyclopedia of genes and genomes) analysis indicated that pyruvate metabolism, carbon fixation, and carbon metabolism were significantly affected in attached cells. Up-regulated proteins such as UTP-glucose-1-phosphate uridylyltransferase, acetyltransferase component of pyruvate dehydrogenase complex, OmpA-like protein, and acetyl-coenzyme A synthetase may be important during initial adhesion. Our findings provide a deeper understanding of the planktonic to sessile transition of marine fouling bacteria.

KEY WORDS: Marine bacteria · *Pseudoalteromonas* · Attachment · iTRAQ · Differentially expressed protein · Biofouling

1. INTRODUCTION

Marine biofouling on the surfaces of submerged artificial structures like vessels, pipelines, bridges, and aquaculture facilities can cause significant economic losses (Yebra et al. 2004, Abioye et al. 2019, Xie et al. 2019). Marine bacteria are usually regarded as early fouling organisms, as they can adhere to and form biofilms on submerged surfaces (Callow & Callow 2002, Abioye et al. 2019, Xie et al. 2019). Marine biofilms themselves can cause serious problems, for example by increasing the drag force on ship hulls, thereby increasing fuel costs and accelerating corrosion (Xu et al. 2017, Hunsucker et al. 2018). Furthermore, marine biofilms can induce the attachment of macrofoulers such as barnacles, tubeworms, and mussels (Bacchetti De Gregoris et al. 2012, Yang et al. 2013, Li et al. 2014, Shikuma et al. 2014, Liang et al. 2019). Thus, investigations into bacterial attachment and biofilm development by bacteria are impor-

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Functional analysis of a tyrosinase gene involved in early larval shell biogenesis in *Crassostrea angulata* and its response to ocean acidification

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ABSTRACT

The formation of the primary shell is a vital process in marine bivalves. Ocean acidification largely influences shell formation. It has been reported that enzymes involved in phenol oxidation, such as tyrosinase and phenoloxidases, participate in the formation of the periostracum. In the present study, we cloned a tyrosinase gene from *Crassostrea angulata* named *Ca-tyrA1*, and its potential function in early larval shell biogenesis was investigated. The *Ca-tyrA1* gene has a full-length cDNA of 2430 bp in size, with an open reading frame of 1896 bp in size, which encodes a 631-amino acid protein that includes a 24-amino acid putative signal peptide. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis revealed that *Ca-tyrA1* transcription mainly occurs at the trochophore stage, and the *Ca-tyrA1* mRNA levels in the 3000 ppm treatment group were significantly upregulated in the early D-veliger larvae. WMISH and electron scanning microscopy analyses showed that the expression of *Ca-tyrA1* occurs at the gastrula stage, thereby sustaining the early D-veliger larvae, and the shape of its signal is saddle-like, similar to that observed under an electron scanning microscope. Furthermore, the RNA interference has shown that the treatment group has a higher deformity rate than that of the control, thereby indicating that *Ca-tyrA1* participates in the biogenesis of the primary shell. In conclusion, and our results indicate that *Ca-tyrA1* plays a vital role in the formation of the larval shell and participates in the response to larval shell damages in *Crassostrea angulata* that were induced by ocean acidification.

1. Introduction

Anthropogenic CO_2 emissions are currently acidifying the world's oceans. Ocean acidification has significantly reduced the pH of seawater in the past century, which in turn has largely impacted marine organisms, particularly calcifying marine invertebrates. Numerous studies on calcifying marine invertebrates have demonstrated that ocean acidification can impact survival, growth, development, and physiology (Talmage and Gobler, 2010; Hofmann et al., 2010; Valerio et al., 2012). Marine calcifiers are particularly vulnerable to ocean acidification because it is more difficult for them to produce calcium carbonate in acidified water (Orr et al., 2005; Fabry et al., 2008). Research on the early development of *Haliotis discus hannai* has shown that ocean acidification results in decreased fertilization and hatching rates, an increase in malformation rate, and a decrease in settlement (Li et al., 2013). Besides, ocean acidification makes it difficult for pteropods to survive because of instability in their biological skeleton, coupled with shell thinning in some foraminifera (Li et al., 2013). It has been reported that *Amphiura filiformis* adapts to an increase in calcification and metabolic rates during ocean acidification; however, as pH decreases, the function of wrist muscles is impaired (Wood et al., 2008). Similar findings have also been reported in *Hemicentrotus pulcherrimus* and *Echinometra mathaei* (Shirayama and Thornton, 2005). These findings indicate that the effect of ocean acidification is widespread in marine animals, and induces marine calcifiers to utilize less calcium carbonate (Watson et al., 2012).

Several mollusks are recognized as economically significant calcifiers. However, shells are vital for most mollusk species. It is reported that the typical adult shells of mollusks are composed of several layers, including the external cuticle layer (periostracum) covering the outer surface of the mollusk shell and inner layers containing the $CaCO_3$ polymorph (calcite and aragonite) (Marin and Luquet, 2004). The shell can be primarily discriminated in gastrulation, and the periostracum is the first observable part of the shell. When larvae develop from gastrulae into early trochophores, an initial shell is formed (Mouëza et al., 2006). To distinguish this from the calcified shell that grows later, Huan et al. (2013) defined this shell as the initial non-calcified shell (InCaS). The InCaS is the first shell during molluscan

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Antifouling Activity towards Mussel by Small-Molecule Compounds from a Strain of *Vibrio alginolyticus* Bacterium Associated with Sea Anemone *Haliplanella* sp.

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Mussels are major fouling organisms causing serious technical and economic problems. In this study, antifouling activity towards mussel was found in three compounds isolated from a marine bacterium associated with the sea anemone *Haliplanella* sp. This bacterial strain, called PE2, was identified as *Vibrio alginolyticus* using morphology, biochemical tests, and phylogenetic analysis based on sequences of 16S rRNA and four housekeeping genes (*rpoD*, *gyrB*, *rctB*, and *toxR*). Three small-molecule compounds (indole, 3-formylindole, and cyclo (Pro-Leu)) were purified from the ethyl acetate extract of *V. alginolyticus* PE2 using column chromatography techniques. They all significantly inhibited byssal thread production of the green mussel *Perna viridis*, with EC_{50} values of 24.45 μg/ml for indole, 50.07 μg/ml for 3-formylindole, and 49.24 μg/ml for cyclo (Pro-Leu). Previous research on the antifouling activity of metabolites from marine bacteria towards mussels is scarce. Indole, 3-formylindole and cyclo (Pro-Leu) also exhibited antifouling activity against settlement of the barnacle *Balanus albicostatus* (EC_{50} values of 8.84, 0.43, and 11.35 μg/ml, respectively) and the marine bacterium *Pseudomonas* sp. (EC_{50} values of 42.68, 69.68, and 39.05 μg/ml, respectively). These results suggested that the three compounds are potentially useful for environmentally friendly mussel control and/or the development of new antifouling additives that are effective against several biofoulers.

Keywords: Marine bacterium, mussel, antifouling, *Vibrio alginolyticus*, compound

Introduction

The accumulation of marine fouling organisms on human-made surfaces in the marine environment poses severe technical and economic problems around the world [1, 2]. Antifouling coatings with metal-based compounds (such as tributyltin and cuprous oxide) and synthetic organic biocides (such as Igarol and diuron) have been widely used to combat marine biofouling [3]. However, these antifoulants have been found to produce toxic effects on non-target species and may pollute the marine environment, leading to bans and regulations on their use in antifouling coatings [3–5]. Thus, environmentally friendly alternatives are urgently needed.

Natural products with antifouling activity from marine organisms are suggested to be a promising source of eco-friendly antifoulants [6, 7]. So far, a large number of secondary metabolites from marine invertebrates and seaweeds have been isolated and demonstrated to have antifouling activity [8]. However, these compounds from marine macroorganisms usually are not available in sufficient quantities for commercial application, and most

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ORIGINAL ARTICLE

Larval settlement and metamorphosis of the invasive biofouler, *Mytilopsis sallei*, in response to ions and neuroactive compounds

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ABSTRACT
In this study, we investigated larval settlement and metamorphosis of the invasive fouler *Mytilopsis sallei* exposed to ions, neurotransmitters and blockers inhibiting their respective actions. Excess K^+ effectively induced larval settlement and metamorphosis, while the voltage-gated potassium channel blocker, TEA, significantly inhibited the K^+ inducing effect, suggesting that a voltage-gated potassium channel may play a role in *M. sallei* settlement and metamorphosis. Excess Ca^{2+} did not induce larval settlement and metamorphosis, while Mg^{2+} and NH_4^+ inhibited both. Among the neurotransmitters, GABA did not induce *M. sallei* larvae to settle and metamorphose at 10^{-6}–10^{-4} M concentrations, while 5×10^{-5}–10^{-4} M L-DOPA (a dopamine precursor), 5×10^{-6}–10^{-4} M dopamine (an epinephrine precursor) and 5×10^{-5}–10^{-4} M epinephrine significantly induced larval settlement and metamorphosis, indicating the presence of an epinephrine biosynthesis pathway in this species and its role in the regulation of larval settlement and metamorphosis. Furthermore, the inducing effect of dopamine on *M. sallei* settlement and metamorphosis was inhibited by SCH23390, a selective D1 dopamine receptor antagonist. Similarly, the inducing effect of epinephrine was inhibited by chlorpromazine, an α_1-adrenergic antagonist, suggesting that the D1 dopamine receptor and α_1-adrenoceptor may play active roles in the processes of settlement and metamorphosis of *M. sallei* larvae. Here, we have shown for the first time the responses of larval settlement and metamorphosis of dreissenid mussels to pharmacologically active compounds. The results provide new insights into the biochemical mechanisms underlying larval settlement and metamorphosis of *M. sallei*, which may be useful to develop effective strategies to control this invasive fouling organism.

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Introduction

Many marine invertebrate species have larvae that remain planktonic for various durations ranging from minutes to months. Then, they settle and metamorphose into benthic juveniles (Hadfield & Paul 2001; Clare 2011). Larval settlement and metamorphosis are not only critical for juvenile growth, but also for adult reproduction (Pawlik 1992), affecting population and community dynamics (Connell 1985). A number of studies have shown that natural and artificial chemical cues influence the larval settlement and metamorphosis of marine invertebrates (Crisp 1984; Pawlik 1992; Hadfield & Paul 2001; Prendergast 2010). However, only a few natural chemical inducers that influence larval settlement and metamorphosis have been fully isolated and chemically characterized (Yvin et al. 1985; Pawlik 1986; Tsukamoto et al. 1999; Swanson et al. 2004; Dreanno et al. 2006; Tebben et al. 2011). Thus, further understanding of the mechanisms that regulate larval settlement and metamorphosis has been limited. Alternatives to natural cues are pharmacological compounds such as agonists and antagonists capable of activating or blocking specific signal transduction pathways. Such studies could also help understanding of the molecular mechanisms associated with larval settlement and metamorphosis (Kimura et al. 2003; Yang et al. 2008, 2011, 2013; Young et al. 2011, 2015; Gohad et al. 2012; Wang et al. 2015). Furthermore, the information is also important for identifying potential inducers and inhibitors of larval settlement and metamorphosis for application in aquaculture (Young et al. 2011; Yang et al. 2013; Wang et al. 2015) and antifouling (Qian et al. 2013; Almeida & Vasconcelos 2015).

A number of compounds have been found to affect the settlement and metamorphosis of marine invertebrates. These include catecholamine, choline

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Embryonic and larval development of the invasive biofouler *Mytilopsis sallei* (Récluz, 1849) (Bivalvia: Dreissenidae)

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ABSTRACT

The marine dreissenid bivalve *Mytilopsis sallei* is a fouling organism that has invaded habitats outside its original range. Understanding its early development will be useful for early detection in the environment, for species identification in ballast water and for development of control strategies targeted at early life stages, which will help us better manage this important invader. The processes of embryogenesis, shell formation and larval development of *M. sallei* are described here for the first time by using light and scanning electron microscopy. Released oocytes are 64 μm in diameter. Fertilized eggs were incubated at 27 ± 1 °C. The trochophore, with an apical tuft and a prototroch, developed by 6.0 ± 2.3 h postfertilization (hpf). At 16.5 ± 4.2 hpf, D-shaped veligers with shell length (SL, mean ± SD) of 87.3 ± 8.2 μm appeared, each possessing a velum and a calcified shell. At 2–3 d postfertilization (dpf), the D-shaped veligers developed into umbonate larvae (SL = 111.9 ± 10.7 μm), the last obligate free-swimming veliger stage. Pediveligers (SL = 232.8 ± 37.1 μm) observed at 6–8 dpf could either swim using their velum or crawl with their foot. Pediveligers settled by secreting byssal threads and metamorphosed to plantigrades (SL = 298.7 ± 45.2 μm) 8–10 dpf. It is noteworthy that the larvae of this invasive bivalve are capable of settlement within 10 d. This is the first detailed study of early shell formation of a species of the family Dreissenidae. Shell field invagination appeared during gastrulation, secreting shell material by expanding over both sides in a saddle-shape during the trochophore stage.

INTRODUCTION

Invasive dreissenid molluscs are ecological and economic nuisances in aquatic ecosystems (Aldridge *et al.*, 2008). They have invaded a variety of habitats outside their native range and are also aggressive biofouling organisms, attaching to submerged hard surfaces by their byssal threads. It has been reported that the dreissenid *Mytilopsis leucophaeata* recently expanded rapidly in Europe (Verween, Vincx & Degraer, 2006) and in some regions has become an economic problem by fouling industrial cooling water systems (Jenner *et al.*, 1998). A population with densities as high as 6.5 million/m^2 was found near power station intakes in the Noordzeekanaal in the Netherlands (Rajagopal, van der Velde & Jenner, 1995). Another invasive dreissenid mussel, *M. trautwineana*, was reported to cause serious fouling problems in shrimp farms on the Caribbean coast of Colombia (Aldridge *et al.*, 2008). The adverse impacts of the invasion of *Dreissena* species on local aquatic ecosystems and water-related structures (e.g. intake pipes) are also well recognized, particularly with zebra mussels (*D. polymorpha*) and quagga mussels (*D. rostriformis bugensis*) (Orlova *et al.*, 2005; Straver, 2009).

Early developmental stages are generally accepted as critical to mussel life cycles. Understanding embryonic and larval development of dreissenid mussels is important for species identification within plankton communities, for investigation of their invasiveness by larval dispersal, settlement and recruitment, and for development of fouling-control strategies. The literature on early development of Dreisseninae has focused on species belonging to the genus *Dreissena*, such as *D. polymorpha* (Meisenheimer, 1901; Ackerman *et al.*, 1994; Lucy, 2006; Cohen, 2008; Harzhauser & Mandic, 2010), and *D. rostriformis bugensis* (Cohen, 2008), while detailed descriptions of early development of *Mytilopsis* is only available for *M. leucophaeata* (Siddall, 1980; Verween Vincx & Degraer, 2010; Kennedy, 2011). Furthermore, most observations of early stages used light microscopy, while scanning electron microscopy (SEM) has not been used to study embryonic and larval development in the Dreisseninae.

The black-striped mussel *M. sallei* (Récluz, 1849), which has a natural Atlantic range, migrated into the Pacific via the Panama Canal (Morton, 1981, 1989). It has now established a firm foothold in the Indo-Pacific Ocean. In China, *M. sallei* was first found in Hong Kong waters (Tolo Harbour) in 1980 (Morton, 1980). This exotic species has now spread along the southeastern coast of mainland China (Cai *et al.*, 2006). *Mytilopsis sallei* is a fecund marine fouling organism. Tan & Morton (2006) found

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Low barnacle fouling on leaves of the mangrove plant *Sonneratia apetala* and possible anti-barnacle defense strategies

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ABSTRACT: A field survey of barnacle fouling on leaves of 5 co-occurring mangrove trees (*Kandelia candel*, *Bruguiera gymnorrhiza*, *Rhizophora stylosa*, *Sonneratia apetala*, and *Avicennia marina*) was conducted in Tongan Bay, southeastern China. The leaves of *S. apetala* were less frequently and less abundantly fouled by barnacles relative to the other mangrove species, suggesting that *S. apetala* may possess anti-barnacle mechanisms. The surface wettability and flexibility of leaves of the 5 investigated species were measured. Except for the lower surface of *A. marina* leaves, the leaf surfaces of all investigated species exhibited low wettability, with water contact angles of 82° to 94°. The leaf flexibility of *S. apetala* was similar to that of *A. marina*, and both were much greater than those of the other 3 species. Leaves of all 5 mangrove species were also subjected to chemical extraction. The extracts of *K. candel*, *S. apetala*, *A. marina*, and *B. gymnorrhiza* showed anti-settlement activities against the barnacle *Balanus albicostatus*, with median effective concentration (EC_{50}) values respectively of 0.85, 10.13, 37.03, and 50.67 µg cm^{-2}. An antifouling compound, identified as oleanolic acid, was isolated from the 2 most active extracts (from *K. candel* and *S. apetala*) via bioassay-guided fractionation. Based on our results, we propose a potential multiple defense strategy of *S. apetala* against barnacle fouling, including low surface wettability, use of an anti-barnacle settlement metabolite of oleanolic acid, and post-settlement detachment of barnacles from its flexible leaves.

KEY WORDS: Mangrove · Barnacle fouling · Antifouling defense · *Sonneratia apetala* · Surface wettability · Oleanolic acid

INTRODUCTION

In the marine environment, surfaces to colonize are often a limited resource, and all natural and man-made surfaces are exposed to the threat of surface fouling by sessile organisms. For living surfaces, the association between epibionts (organisms growing attached to animate surfaces) and basibionts (substrate organisms) is termed epibiosis (Wahl 1989, 2009, Harder 2009) and is among the closest known interspecific associations. The ecological consequences of marine epibiosis can be positive and negative for the organisms involved (Harder 2009, Wahl 2009). Epibiont fouling can cause beneficial effects to the basibiont, such as camouflage and nutrient flow from the epibiont (Feifarek 1987, Thevanathan et al. 2000). However, epibiosis more often appears to be disadvantageous for basibionts. Epibionts can increase weight, drag, and surface friction of the host; cause mechanical and chemical damage to the host; compete with the host for nutrients; interfere with vital processes such as gas exchange, excretion, and sensing; and obstruct host feeding (Wahl 1989, 2009, Bers & Wahl 2004, Harder 2009). Furthermore,

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Antifouling activity of marine sessile organisms from China against barnacle settlement

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The antifouling activity of a series of hexane, ethyl acetate, ethanol and aqueous extracts from 11 species of marine sessile organisms collected from the south-east coast of China was investigated. Settlement inhibition of cyprid larvae of the barnacle Balanus albicostatus *was used to evaluate their antifouling efficacy. Screening of the 44 extracts showed antifouling activity in 90.9% of the hexane extracts followed by 90.9% of the ethyl acetate, 72.7% of the ethanol and 36.4% of the aqueous extracts. The hexane extracts of* Tubularia mesembryanthemum, Notarcus leachii cirrosus *and* Styela canopus, *the ethyl acetate extracts of* Bugula neritina *and* N. leachii cirrosus, and the ethanol extracts of B. neritina *and* Anthopleura sp. *were the most active in inhibiting the settlement of* B. albicostatus, *with EC_{50} values all below 50 μg/ml. At least one of the four extracts of each tested species exhibited antifouling activity, suggesting that all 11 marine sessile organisms contained antifouling substances and they may have evolved chemical defences against biofouling on their surfaces.*

Keywords: antifouling, marine sessile organisms, extracts, settlement, barnacle

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INTRODUCTION

Man-made structures in the marine environment, such as ship hulls, cooling systems pipes of power plants and aquaculture equipment, are exposed to surface colonization by marine fouling organisms. This poses serious threats to the safe and efficient operation of these marine structures and constitutes a global technical and economic problem (Richmond & Seed, 1991; Rittschof, 2000; Townsin, 2003; Yebra *et al.*, 2004; Pérez *et al.*, 2006). Although coating surfaces with metal-based antifouling paints, in particular those containing organotins, is the most effective solution to marine biofouling (Alberte *et al.*, 1992; Clare, 1996; Yebra *et al.*, 2004), environmental and human health problems associated with the use of toxic antifoulants (Ellis, 1991; Lau, 1991; Cardwell *et al.*, 1999; Guerin *et al.*, 2007) have led to regulations for, or bans on, their use in a number of countries (Dalley, 1989; Rittschof, 2001; van Wezel & van Wlaardingen, 2004). This makes the development of non-toxic or biodegradable alternatives a necessity (Clare *et al.*, 1992; Fusetani, 2004; Yebra *et al.*, 2004).

Marine sessile organisms are also susceptible to surface biofouling. Epibionts can decrease the fitness or even lead to the death of the host by increasing weight, drag and surface friction; reducing elasticity; interfering with vital processes such as gas exchange, nutrient absorption, excretion and sensing; and enhancing susceptibility to predation (Witman & Suchanek, 1984; Wahl, 1989; Williams & Seed, 1992). Consequently antifouling defence mechanisms are expected to have evolved in some less-fouled marine sessile organisms (Guenther *et al.*, 2007). In fact, many secondary metabolites with antifouling activity are found in many marine invertebrates and seaweeds, suggesting the presence of a chemical defence against epibionts (Clare, 1996; Fusetani, 1998, 2004; Armstrong *et al.*, 2000; Rittschof, 2000, 2001; Steinberg *et al.*, 2001). These naturally occurring antifouling compounds could provide a potential source of environmentally friendly alternatives to toxic antifoulants. Therefore, research on the antifouling activity of secondary metabolites or extracts from such marine sessile organisms could provide important information not only for understanding the chemical mediation of host–epibiont interactions, but also for non-toxic fouling control in marine technology. Thus, in recent years, a large number of studies have focused on screening compounds and extracts from marine organisms for their antifouling activity. In some of these studies, individual compounds are isolated, structurally characterized and tested for their antifouling activity (Okino *et al.*, 1996; Tsukamoto *et al.*, 1997; Cho *et al.*, 2005). On the other hand, crude extracts are also used to evaluate the antifouling activity of marine organisms (Willemsen, 1994; Devi *et al.*, 1998; Cho *et al.*, 2001; Wilsanand *et al.*, 2001; Bhosale *et al.*, 2002; Hellio *et al.*, 2004; Bers *et al.*, 2006). The use of crude extracts is very important and should not be discounted, since it provides a method for the widest possible screening for antifouling substances and because synergism may exist between the different components of an extract.

Therefore, the present study investigated the antifouling potential of crude extracts from various marine sessile organisms. The species chosen all occur abundantly along the

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Settlement and metamorphosis of *Styela canopus* Savigny larvae in response to some neurotransmitters and thyroxin

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Abstract

The larvae of ascidian *Styela canopus* Savigny were treated with epinephrine, norepinephrine, L-DOPA, GABA and thyroxin to test the ability of these compounds to induce or inhibit larval settlement and metamorphosis. The results showed that epinephrine, norepinephrine and L-DOPA at the concentration of 1 μmol/dm^3 induced larval settlement and metamorphosis in *S. canopus*, with short exposure (1 h) to 1 μmol/dm^3 of L-DOPA inducing rapid settlement. In contrast, GABA at the concentrations of 0.1 ~ 100.0 μmol/dm^3 significantly inhibited the settlement and metamorphosis of *S. canopus* larvae. In addition, thyroxin at 1 ~ 50 μg/dm^3 had no effect on larval settlement and metamorphosis in *S. canopus*. These results suggest the importance of neurotransmitters in the settlement and metamorphosis of *S. canopus* larvae.

Key words: *Styela canopus*, larvae, settlement, metamorphosis, neurotransmitter, thyroxin

1 Introduction

As important steps in the life cycles of most marine benthos, settlement and metamorphosis of their planktonic larvae influence directly the population distribution and fluctuation of benthos. Research on larval settlement and metamorphosis of marine benthos, related to aquaculture (Ke et al., 2000; Liu et al., 1998) and marine antifouling (Huang, Feng, et al., 2003), is popular in marine biology. The influences of various chemicals on larval settlement and metamorphosis have been studied in many species of marine benthos. Among these investigations, the inductive effects of various neurotransmitters on the settlement and metamorphosis of larvae have been found in many marine benthos, of which the most outstanding ones were choline on *Phestilla sibogae* (Hirata and Hadfield, 1986), L-DOPA on *Crassostrea gigas* (Coon et al., 1985) and GABA on *Haliotis rufescen* (Morse et al., 1979).

Ascidians (Urochordata) are an important marine benthic organism and have special significance in animal evolution and classification because the ascidian larva is considered a basic model of vertebrate morphogenesis (Huang, Ke, et al., 2003). To date, the mechanisms regulating the settlement and

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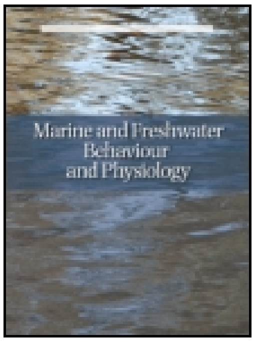

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The influence of temperature and light on larval pre-settlement metamorphosis: a study of the effects of environmental factors on pre-settlement metamorphosis of the solitary ascidian *Styela canopus*

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