Marine Environmental Research 113 (2016) 134–140

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Antifouling activity of green-synthesized 7-hydroxy-4-methylcoumarin

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ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form
25 November 2015
Accepted 27 November 2015
Available online 2 December 2015

Keywords:
Biofouling
antifouling coatings
coumarins
green chemistry

ABSTRACT

In the search for new environmental-friendly antifoulants for replace metallic biocides, 7-hydroxy-4-methylcoumarin was synthesized according to green chemistry procedures. This compound was characterized by current organic analysis and its antifouling properties were firstly evaluated on the bivalve *Mytilus edulis platensis* in the laboratory. In the second stage, a soluble matrix antifouling coating formulated with this compound was assayed in marine environment. Laboratory experiments showed that 7-hydroxy-4-methylcoumarin was effective in inhibiting both the settlement as well as the byssogenesis of mussels. In addition, after exposure time in the sea, painted panels containing this compound showed strong antifouling effect on conspicuous species of the fouling community of Mar el Plata harbor. In conclusion, green-synthesized coumarin could be a suitable antifoulant candidate for marine protective coatings.

1. Introduction

Competition for space is intense in benthic marine environments; hence, all submerged surfaces are rapidly colonized by invertebrates and algae which generate serious threats to the safe and efficient operation of vessels worldwide (Hellio et al., 2005; Davidson et al., 2010; Dobretsov et al., 2013; Ferguson et al., 2013).

This community attached to human-made surfaces is called 'biofouling', and biofoulers include microfoulers such as marine bacteria, algae, and protozoa and macrofoulers as barnacles, tubeworms, bryozoans and ascidians (Callow and Callow, 2002; Dobretsov et al., 2006; Wahl, 1989).

Marine biofouling has long been acknowledged as a persistent problem with severe consequences both in the economic and ecological point of view (Callow and Callow, 2011). There is a substantial industrial and commercial interest in controlling the biofouling process because it leads to enormous economic losses for maritime structures. They increase the weight, drag, and surface corrosion of ships, and lead to huge costs in maintaining mariculture systems (Chambers et al., 2006). In this sense, estimates suggest that fuel consumption would increase by as much as 40% if no antifouling measures were taken (Yebra et al., 2004).

One of the most common methods to minimize the impacts of foulers is the coating of the ship hull with antifouling compounds.

A remarkable number of toxic materials as copper, lead, mercury and arsenic were used to control fouling organisms until organotins were introduced in the 1960's (Flemming and Trevors, 1989; Iwao, 2003). Undoubtedly, organotins (such as tributyltin, TBT) were the most effective antifouling agents known, but also among the most toxic biocides because they are not readily degraded in the natural environment (Alzieu et al., 1989; Claisse and Alzieu, 1993; Evans et al., 1995; Omae, 2003; Qian et al., 2010). This led the International Maritime Organization (IMO) to prohibit their application to ships, since 2008 (IMO, 2007). Yet, antifouling coatings are highly toxic and nonspecific because they act on both target and non-target organisms (Yebra et al., 2004).

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http://dx.doi.org/10.1016/j.marenvres.2015.11.010
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A more environmentally friendly antifouling strategy has focused on the characterization and development of products that are based on the chemical defenses of sessile marine organisms that maintain their body surfaces free of fouling. A variety of new natural products have been isolated from various marine sources in the past decades. New natural products have provided key structures and promising compounds with the potential to be used as new antifoulants. In this sense, the majority of natural products were identified as terpenoids, steroids, carotenoids, phenolics, furanones, alkaloids, peptides and lactones (Raveendran and Limna Mol, 2009). They have been isolated mainly from microorganisms, sponges, soft-corals, bryozoans, tunicates, and also terrestrial plants (Dobrestov and Qian, 2002; Harder et al., 2003; Hentschel et al., 2001; Pérez et al., 2014).

Plants are useful sources of molecules for the development of new pharmaceutical products. Coumarins are one of the most important secondary metabolites of plants and known as naturally occurring benzo-α-pyrone derivatives from the metabolism of phenylalanine. They are found at high levels in some essential oils, particularly cinnamon bark oil, cassia leaf oil and lavender oil. Coumarin is also found in fruits (e.g. bilberry, cloudberry), green tea and other foods such as chicory (Lake, 1999). Many kinds of coumarins such as furocoumarins and pyranocoumarins arise from the biosynthetic pathway as being the substitution of the coumarin ring following by some steps such as prenylation, cyclization or glycosylation (Tosun, 2012).

Several natural products with a coumarinic moiety have been reported to have multiple biological activities. Coumarins exhibit many bioactivities including anticoagulant, estrogenic, dermal photosensitizing activity, antimicrobial, antifungal, vasodilator, moluscicidal, anthelmintic, sedative and hypnotic, analgesic and hypothermal activity (Asma'a et al., 2012; Céspedes et al., 2006; Dini et al., 1992; Edelson, 1988; Nitiema et al., 2012; Sardari et al., 2000; Shakeel-u-Rehman et al., 2010; Soine, 1964). Particularly, antibacterial activity was attributed to the coumarin ring by inhibition of bacterial nucleic acid synthesis (Rosselli et al., 2007). Also, it was found that coumarins reduce biofilm formation via quorum sensing inhibition (Gutiérrez-Barranquero et al., 2015; Lee et al., 2014). Even though some coumarins are toxic compounds, 7-hydroxy-4-methylcoumarin is a safe compound used as active ingredient in several approved drugs (Benitez et al., 2013; Nagy et al., 2015). In the pharmaceutical industry 7-hydroxy-4-methylcoumarin is known by the name of hymecromone. In humans, hymecromone has been reported as glucuronosyltransferase inhibitor in concentrations above 42.5 microM (Hanioka et al., 2008). On relevant worldwide toxicity database as EPA or Canada Domestic Substance List, hymecromone is cataloged as no bioaccumulative, no inherently toxic to aquatic organisms and no persistent (EPA, 2015). Furthermore, it is noteworthy that coumarins could be biotransformed by several environmental bacteria, fungi and yeasts. On the other hand, it is well known that some bacteria, as *Arthrobacter* sp. and *Pseudomonas* sp., and fungus as *Aspergillus* spp., are involved in degradation of coumarins (Aguirre-Pranzoni et al., 2011; Kunc, 1974; Levy and Frost, 1966; Marumoto and Miyazawa, 2011; Nigam, 2013; Sima Sariaslani and Rosazza, 1983). In spite of many natural coumarins could be isolated from higher plants and microorganisms, total synthesis in the laboratory of this compound by means of 'green chemistry' techniques is also possible. Green Chemistry involves a series of twelve principles that leads to safer processes, reactants, techniques and products. Some of theses principles states that a green chemistry must lead to: less hazardous chemical syntheses, designing safer chemicals, safer solvents and auxiliaries, reduce derivatives, reduce derivatives, and design for degradation, among others. Summarizing, Green Chemistry is an environmentally-friendly alternative to synthesize new safe antifouling compounds.

Little information is available on the activity of coumarins on fouling organisms referred only to laboratory tests (Iyapparaj et al., 2012; Kim et al., 2013; Wang et al., 2013). There are some investigations concerning to the use of coumarin in bioactive coatings in which it has been determined that coumarin moiety play a very important role as antimicrobial agent (Jaiswal et al., 2013; Patel et al., 2008, 2011; Srivastava et al., 2012). However, the use of coumarins in antifouling coatings has been an issue so far unexplored. Laboratory assays do not provide sufficient information with respect to the field performance of antifouling paints. The range of factors determining the antifouling performance in the field is complex but field trails are realistic (Briand, 2009; Dhams and Hellio, 2009).

The aim of this study was to evaluate the potential antifouling properties of a green lab-synthesized compound, 7-hydroxy-4-methylcoumarin (Fig. 1) on the bivalve *Mytilus edulis platensis* in the laboratory and in field trails included in antifouling paints.

2. Materials and methods

2.1. Fouling organisms

A typical species of fouling organisms at Mar del Plata harbor (Argentine), *M. edulis platensis* was chosen for the experiments. Mussels were collected together with their rock substratum from intertidal rocks in Playa Chica (38°08′17″S, 57°31′18″W). In the laboratory, individuals were then disaggregated and conditioned in artificial seawater (ASTM D1141/75), pH 8.2–8.3, salinity 33–35‰, temperature 22 ± 2 °C, with suitable aeration and natural light.

2.2. Optimized procedure to the synthesis of 7-hydroxy-4-methylcoumarin

Chemicals were purchased from Aldrich and Fluka chemical companies and were freshly used after purification by standard procedures (distillation and recrystallization). All the reactions were monitored by TLC on precoated silica gel plates (254 mm). All the yields were calculated from crystallized products. The product was identified by comparison of physical data (mp, TLC and NMR) with those reported or with these of authentic sample prepared by the respective conventional methods using sulfuric acid as catalyst. Melting point of the compound was determined in sealed capillary tube and is uncorrected. The 1H NMR and 13C NMR spectra were obtained on an NMR Bruker Advance DPX 400 spectrometer as d6-DMSO solutions, and the chemical shifts were expressed in d units with Me4Si (TMS) as the internal standard. The catalyst $H_{14}(NaP_5W_{29}MoO_{110})$ was synthesized according to a procedure of the literature (Pasquale et al., 2013).

7-hydroxy-4-methylcoumarin was synthesized using green chemistry methodologies (Heravi et al., 2007; Romanelli et al., 2004) by means of Pechmann reaction from a mixture of resorcinol (10 mmol) and ethyl acetoacetate (10 mmol). The mixture was stirred at 130 °C in the presence of bulk $H_{14}NaP_5W_{30}O_{110}$ Preyssler heteropolyacid (1% mmol) for 1 h in a round bottom flask, which

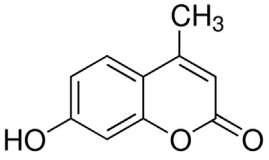

Fig. 1. 7-hydroxy-4-methylcoumarin structure.

was equipped with a condenser and immersed in an oil bath. The reaction mixture was extracted with hot toluene (3 × 5 mL), the solution was concentrated and the crude product recrystallized from methanol. Pure product was characterized by its melting point, ^{13}C NMR, ^{1}H NMR, IR and MS spectra.

2.3. Preparation of coumarin solutions

A stock solution was prepared by dissolving 300 mg of 7-hydroxy-4-methylcoumarin in 10 mL of methanol. From this stock solution (30 mg/mL) eight dilutions were obtained: 3.0; 2.1; 1.4; 0.9; 3.6; 0.3; 0.03 and 0.003 mg/mL. Then, 1 mL from each solution was pippeted out, placed in a Petri dish 9 cm in diameter, and allowed to evaporate the organic solvent at room temperature.

2.4. Laboratory tests

2.4.1. General procedure

Laboratory studies were conducted to evaluate thread production at different coumarin concentrations. Mussel bioassay was done by following the method of Harada et al. (1984) and Ina et al. (1989) modified one.

Tests for antifouling activity were conducted using six healthy mussels (1.0–1.5 cm length) fixed with two-component epoxy-adhesive (Poxipol®) to each Petri dish. The compound was assayed over a concentration of 0.047, 0.47, 4.7, 9.4, 14.0, 22.0, 33.0 and 47.0 μg/cm^2. Then, each vessel was filled with 30 mL of artificial seawater. For controls, 1 mL of organic solvent was added to Petri dishes and allowed to evaporate. After that, six mussels and 30 mL of artificial seawater were conditioned.

Three replicates were set up for each of the treatment groups and for controls. Mussels were not fed and seawater was not changed during the experiment. All test Petri dishes were incubated at a temperature of 14–18 °C in darkness.

2.4.2. Byssal thread inhibition

This experiment was conducted to determine coumarin concentration necessary to inhibit fifty percent of byssal thread number after 24 h exposure. After this period of time, byssal threads were stained with fucsine to enhance visualization and counting under stereomicroscope.

For recovery tests, all individuals from each coumarin concentration and replicas were transferred to clean artificial seawater and kept there for 24 h. Individuals not attached were transferred directly, and in the case of attached individuals byssuses were previously cut.

After this period, number of byssal thread produced and attachment were observed. Then, percentage of recovery was calculated. Data from these experiments contribute to know the degree of toxicity of the compound.

2.4.3. Attachment assay

This experiment investigated the number of settled and not-settled mussels at different coumarin concentrations after 24 h exposition. The effective concentration at which 50% of mussels showed inhibition of attachment was determined as EC_{50}.

2.5. Field trials

2.5.1. Antifouling paints

Colophony (WW rosin) was used as binder and oleic acid as plasticizer. The paint was prepared in a laboratory scale ball mill (1.0 L jars); the operating conditions of the ball mill were chosen so as to achieve an efficient dispersion. Antifouling paint was prepared by dissolution of colophony and oleic acid in a xylene/methyl isobutyl ketone mixture (1:1) using a high-speed disperser; the ball mill was then loaded with this mixture ('vehicle') and pigments (zinc oxide and calcium carbonate), and dispersed for 24 h. Subsequently, paint was filtered and fractionated in two portions, one of which was used as a negative control and the remaining as treatment.

For treatment, 7-hydroxy-4-methylcoumarin previously dissolved in 1 mL of MeOH, was incorporated into the matrix paint at 2% Wt and dispersed during 1 h (Table 1).

Antifouling paints were applied on acrylic tiles (4 × 12 cm), which were previously sandblasted and degreased with toluene. Four layers of paint were applied leaving a drying time of 24 h between each coat to obtain a final dry thickness of 75 ± 5 μm. Coated panels were submerged in a marina in Mar del Plata harbor. Uncoated plates and plates coated with base paint, i.e., paint without coumarin, were considered as controls. Field experiments were evaluated after 45 and 90 days exposure in the sea during the summer season (December–March) representing a period with intense settlement of organisms. The settlement of fouling organisms was estimated as percentage cover on paints using a dot grid estimate method (Foster et al., 1991). All tests were performed in triplicate.

2.6. Statistical analysis

All statistical analyses were performed with Statistica 8 software. The normality assumption was verified with the Shapiro Wilk's test (Shapiro and Wilk, 1965) and variance homogeneity with Levene's test. The differences between treatments and control were determined by one-way analysis of variance (ANOVA) followed by LSD post hoc test. Differences were considered to be significant at $p < 0.05$.

Estimations of EC_{50} and reduction of fifty percent of byssal thread number were calculated through Probit analysis.

3. Results

3.1. Characterization data of 7-hydroxy-4-methylcoumari

The procedure described above provides a useful, clean and fast alternative for the preparation of 7-hydroxy-4-methylcoumarin, obtaining 92% yield of pure product. The reaction time could be reduced among 7–8 times compared to the classical methods.

mp: 186–187 °C (lit. p.f.:185–187 °C (Bahekar and Dhinde, 2004))

^{13}C NMR (DMSO-d_6, 100 MHz): δ 162.5 (C-7), 161.8 (C-2), 155.3 (C-8a), 155.0 (C-4), 127.6 (C-5), 114.3 (C-4a), 113.1 (C-6), 110.9 (C-3), 103.2 (C-8), 19.0 (CH_3).

^{1}H NMR (DMSO d_6, 400 MHz): δ 10.53 (1H, s, OH), 7.45 (1H, d, *J*: 8.8, H-5), 6.70 (1H, dd, *J*: 8.8, *J*: 2.3, H-6), 6.58 (1H, d, *J*: 2.3, H-8), 5.99 (1H, d, *J*: 1.1, H-3), 2.24 (3H, d, *J*: 1.0, CH_3).

Mass spectra: m/z: 176 (M^+, 70%), 148 (100%), 147 (49%), 92 (15%).

IR spectra (KBr, cm^{-1}): 3080, 1690, 1610.

Table 1
Paint composition expressed as % by Wt.

Components	Base paint (BP)	Antifouling paint (7 HC)
Zinc oxide	43.9	41.9
Calcium carbonate	14.1	14.1
Colophony	17.1	17.1
Oleic acid	2.9	2.9
7-Hydroxy-4-methylcoumarin	–	2.0
Xylene/MIBK (1:1)	22.0	22.0

3.2. Laboratory test

3.2.1. Byssal thread inhibition

Mussels are one of the major fouling organisms settling on man made or natural surfaces and can be used as bioindicators to study the antifouling potency against the macroorganisms (Iyapparaj et al., 2012). Adult mussels are convenient test organisms based on several of their biological characteristics, including their capacity for sensing chemical and physical characteristics of a substratum using the foot, secreting byssal threads for attachment to a surface, releasing byssal threads from unfavorable surfaces, and reattachment by secretion of new byssal threads (Oldham, 1930; Ayala et al., 2006).

Coumarins significantly inhibited byssal thread attachment in relation to control exhibiting strong antifouling activity. Laboratory experiments showed that a reduction of fifty percent in byssal thread number was obtained at a concentration of 0.157 μg/cm^2 ($p < 0.05$). However, byssogenesis was affected from concentrations as low as 0.047 μg/cm^2 (Fig. 2).

Recovery test demonstrated that byssal production was not affected by exposition to coumarin in most concentrations. A comparison made between treated and control mussels showed that there are no significant differences in byssal thread number, i.e., mussels recovered immediately after exposure to the compound indicating that the presence of coumarin had not caused any irreversible damage (Fig. 3). In contrast, byssogenesis was markedly affected above a concentration of 22 μg/cm^2.

3.2.2. Attachment assay

Byssus secretion is an important trait that ensures firm attachment to sites suitable for settlement. The effective coumarin concentration for fifty percent inhibition of mussel attachment (EC_{50}) was 11 μg/cm^2 (Fig. 4).

As it has been indicated, there are few reports about the antifouling activity of coumarins, and in all cases the tests conducted were only at laboratory scale (Iyapparaj et al., 2012; Kim et al., 2013; Wang et al., 2013). In this sense, it was found that methanolic extract containing coumarins obtained from marine macroalgae, was able to inhibit completely the byssal thread production and attachment of brown mussel *Perna indica* (Iyapparaj et al., 2012).

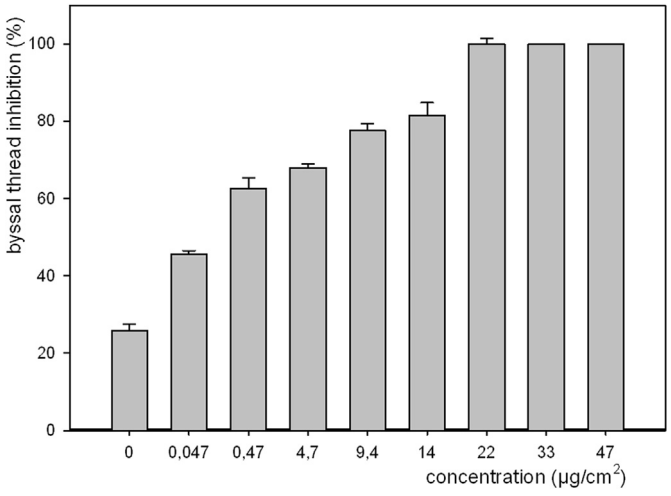

Fig. 2. Percentage of byssal thread inhibition after 24 h exposure. Bars = mean ± SE.

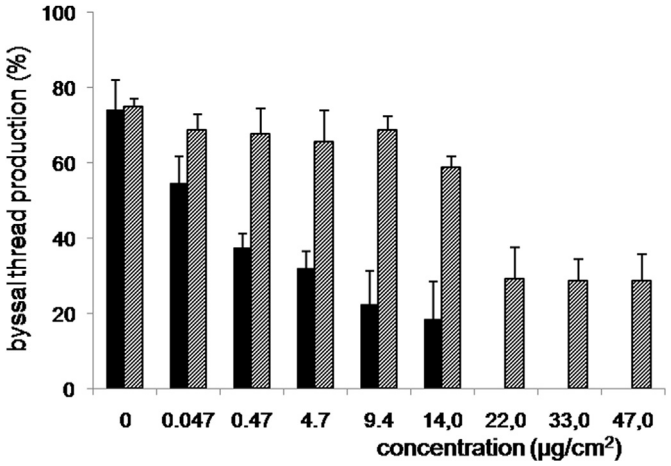

Fig. 3. Byssal thread percentage secreted for each coumarin concentration after 24 h exposition (black bars). Byssal thread percentage secreted in recovery tests (grey bars). Bars = mean ± SE.

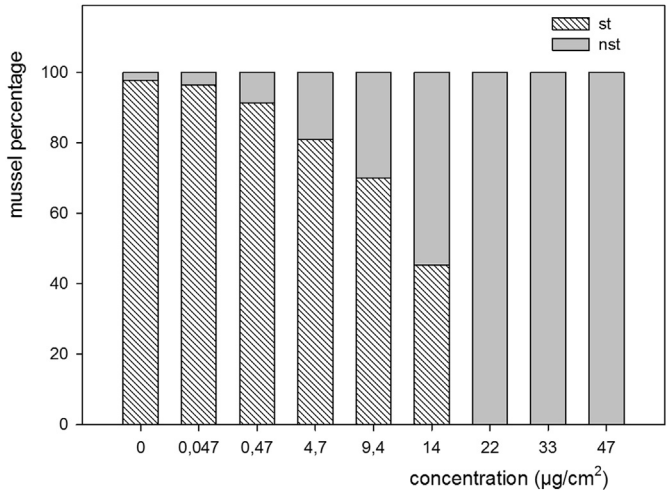

Fig. 4. Mussel attachment percentage for each coumarin concentration. st = settled mussels, nst = non-settled mussels.

3.3. Field trials

The main macro and microfouling species of Mar del Plata harbor were strongly affected by all coatings containing coumarins.

As shown in Fig. 5, after 45 days exposure significant differences were detected on the settlement of the algae *Enteromorpha intestinalis* and *Ectocarpus* sp., bryozoan colonies (*Bugula stolonifera*), tube-builders polychaetes (*Polydora* sp., *Hydroides elegans*), and solitary (*Ciona intestinalis*) and colonial ascidians (*Botryllus* sp.) ($p < 0.05$). Also, diatoms were particularly affected by coumarin based antifouling paints, i.e., *Nitszchia* sp., *Melosira* sp., *Skeletonema costatum*, *Pleurosigma* sp. and *Thalassiotrix* sp. were virtually absent on painted panels (Fig. 6). This performance maintained after 90 days exposure for all species except for *Botryllus* sp. ($p < 0.05$) (Figs. 7 and 8).

These results agree with those observed for other coumarins isolated from higher plants, e.g., coumarins isolated from the cinnamon tree *Cinnamomum loureiroi* were effective in inhibiting the settlement of the seaweed *Ulva pertusa* and diatom *Navicula annexa* (Kim et al., 2013). Also, coumarins from the herb *Cnidium monnieri* have shown a marked antisettlement effect on the bryozoan *Bugula*

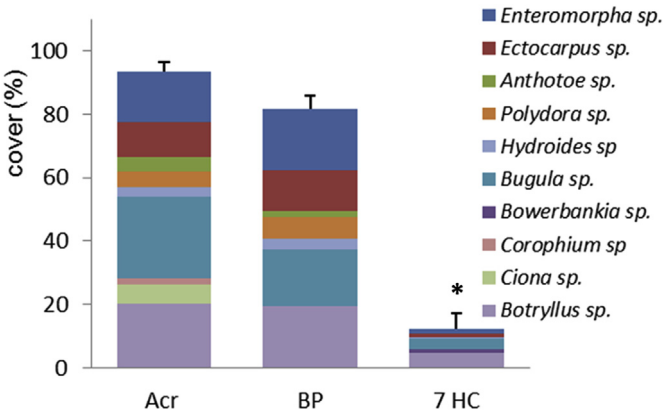

Fig. 5. Macrofouling percentage cover on painted panels vs. controls, 45 days exposure. Acr: acrylic panel, BP: base paint, 7 HC: antifouling paint containing 7-hydroxy-4-methylcoumarin. Bars = mean ± SE, (*) significant differences, $p < 0.05$.

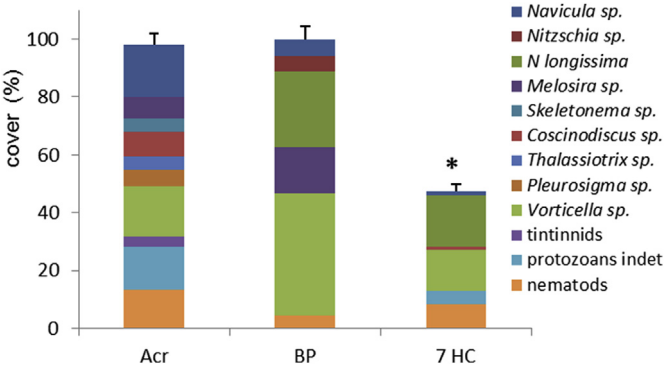

Fig. 6. Microfouling percentage cover on painted panels vs. controls, 45 days exposure. Acr: acrylic panel, BP: base paint, 7 HC: antifouling paint containing 7-hydroxy-4-methylcoumarin. Bars = mean ± SE, (*) significant differences, $p < 0.05$.

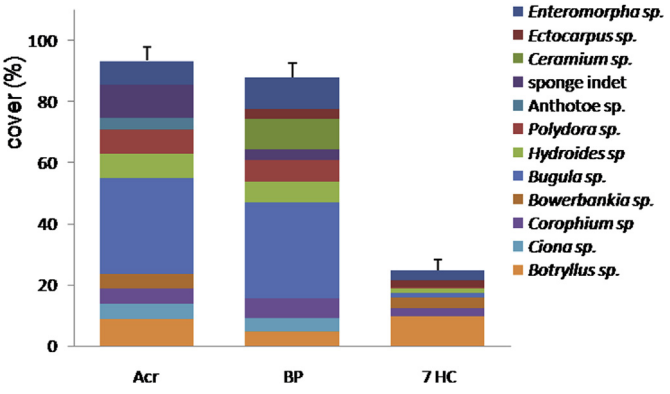

Fig. 7. Macrofouling percentage cover on painted panels vs. controls, 90 days exposure. Acr: acrylic panel, BP: base paint, 7 HC: antifouling paint containing 7-hydroxy-4-methylcoumarin. Bars = mean ± SE, (*) significant differences, $p < 0.05$.

neritina and the hard fouler *Balanus albicostatus* (Wang et al., 2013). Conversely, hard fouling species such as *Balanus amphitrite* have not been recruited in our field trials.

In spite of *M. edulis platensis* is a common fouler at Mar del Plata harbor, they did not colonize experimental panels because they recruit seasonally since August to December with a maximum peak in October (Penchaszadeh, 1974; Oyarzún et al., 2011).

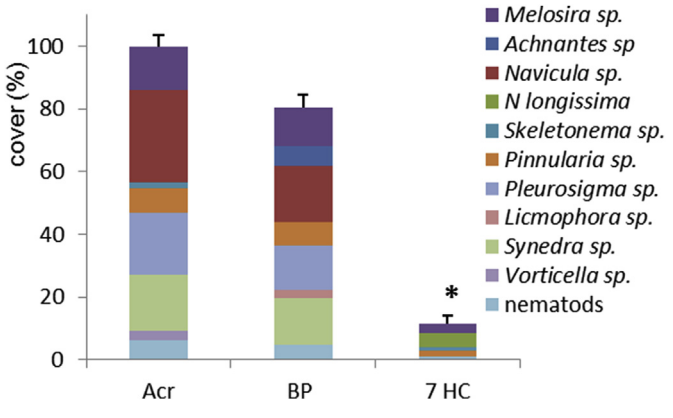

Fig. 8. Microfouling percentage cover on painted panels vs. controls, 90 days exposure. Acr: acrylic panel, BP: base paint, 7 HC: antifouling paint containing 7-hydroxy-4-methylcoumarin. Bars = mean ± SE, (*) significant differences, $p < 0.05$.

4. Discussion

Coumarin properties were intensely studied in relationship with molecular structure. In this sense, structure–activity relationships (SAR) have revealed that O-substitutions are essential for antifungal activity (de Araújo et al., 2013) and position of polar (OH) and less polar (OMe, Me) functions are associated with antibacterial activity (de Souza et al., 2005). A typical characteristic of coumarin molecule is its lipophilic property and this has been demonstrated to strongly influence bioactivities in many cases (Gupta, 2001; Siddiqui et al., 1999; Xu, 2009). One of the explanations is that the compound can penetrate the cell by passive diffusion more easily when the molecule is more lipophilic, and consequently, can lead its bioactivity at reduced concentration compared to less lipophilic molecules (Siddiqui et al., 1999). Kayser and Kolodziej (1999) suggested that fairly high antibacterial activity of coumarin per se is due to both its lipophilic character and planar molecular structure, which contribute in penetration through bacterial cell membrane or cell walls. As a consequence, coumarin affects biofilm formation and the progress of fouling sequence.

Furthermore, its high lipophilicity would reduce its solubility in seawater and improve its incorporation in a potential antifouling paint. It is also a favorable property for antifouling industry as lipophilic compounds are easier to be incorporated into paints and leach out from the paints more slowly than hydrophilic compounds.

Among the invertebrate species that cause biofouling problems, mussels are one of the major fouling organisms settling on man made or natural surfaces. The findings of the present results clearly manifested that coumarin has actively participated in byssal thread inhibition and settlement of mussel *Mytilus*.

The antifouling activity of green synthesized coumarin directly incorporated in a soluble matrix coating was examined in harbor trials. It can be concluded from this study that coumarin displayed clear antifouling properties against micro and macrofoulers. Compared to control paint, antifouling coatings containing coumarin exhibited much reduced fouling coverage for 90 days of immersion. In spite of its high performance against main fouling organisms along the experiment, antifouling coating was not effective to avoid colonial ascidians settlement.

Bryan et al. (2003) suggested that colonial ascidians possess compounds within their tissues that aid in the prevention of surface fouling. These compounds appear to be effective at both preventing growth of marine bacteria and attachment of common fouling macroorganisms. In addition, Wahl et al. (1994) isolated

biologically active polar and non-polar compounds from water collected near the surfaces of tunicates. Further research would be necessary to study possible synergisms between coumarins and secondary metabolites from ascidians.

Pechmann reaction is the most used method for preparing 4-substituted coumarins since it proceeds from very simple starting materials, phenols and β-ketoesters or α, β-unsaturated carboxylic acids. The reaction involves acidic catalysis, and good yields of coumarins can often be obtained. However, rough quantities of mineral acid are usually required in the classical preparations, leading to increase the environmental pollution. For example, a well-established textbook of practical organic chemistry specifies the use of 1.1 L of concentrated H_2SO_4 for preparing 1 mol of 7-hydroxy-4-methylcoumarin by the Pechmann reaction (Romanelli et al., 2004). In recent years, considerable emphasis has been placed on decreasing the environmental impact of industrial chemical processes. The broad utility of heteropolyacids (HPAs) as acid and oxidative catalysts in solution as well as in the solid state for various industrial processes has been demonstrated for a wide variety of synthetically useful transformations of organic substrates. HPAs are strong Bronsted acids composed of heteropolyanions and protons as the counter cations. They are stronger than many conventional solid acids such as mixed oxides, zeolites and so forth (Heravi et al., 2007).

The use of $H_{14}NaP_5W_{30}O_{110}$ Preyssler heteropolyacid as catalyst combined with 'solvent-free' conditions is a promising alternative for the synthesis of coumarin derivatives. The outstanding features of Preyssler's anion are availability, non-toxicity and reusability. 'Green advantages' of the described procedure are the low formation of wastes, no requiring for the use of adsorbents, and principally, the replacement of corrosive mineral acids. Reduction of reaction time and solvent-free procedure are also interesting features from an environmental and economical point of view.

The organic synthesis allows obtaining a large number of bioactive molecules or enhancing the activity of known compounds on the basis of knowledge of the "key moieties" present in the natural product which may be involved in the antifouling activity. In this context, total synthesis in the laboratory of a coumarin family by means of 'green chemistry' techniques takes an important role from the standpoint of environmental care for new antifoulants.

The development of suitable laboratory syntheses of these molecules is important because it is often practically impossible to extract enough of the chemicals from living organisms to meet the needs of industry, e.g. antifouling coatings. Greener alternatives to biocide-based technologies are therefore urgently sought by the marine coatings industry, and there is considerable interest in developing biocide-free coatings.

In conclusion, *7-hydroxy-4-methylcoumarin* meets many criteria to be a good candidate for low-toxic antifouling additives for marine paints.

Acknowledgments

We thank to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CICPBA) for their financial support. We also wish to thank the Club de Motonáutica of Mar del Plata for permission to use their marine testing site. Special thanks to Dr Laura Schejter for having provided the adult mussels.

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