Biofouling
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454511

Reversible effect of potassium sorbate on Balanus amphitrite larvae. Potential use as antifoulant

Guillermo Blustein [ab]; Miriam Pérez [ab]; Mónica García [a]; Mirta Stupak [a]; Claudio Cerruti [c]
[a] CIDEPINT (Centro de Investigación y Desarrollo en Tecnología de Pinturas), La Plata, Argentina [b] Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina [c] Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

First Published on: 22 May 2009

To cite this Article Blustein, Guillermo, Pérez, Miriam, García, Mónica, Stupak, Mirta and Cerruti, Claudio(2009)'Reversible effect of potassium sorbate on Balanus amphitrite larvae. Potential use as antifoulant',Biofouling,25:6,573 — 580
To link to this Article: DOI: 10.1080/08927010902995572
URL: http://dx.doi.org/10.1080/08927010902995572

Biofouling
Vol. 25, No. 6, August 2009, 573–580

Taylor & Francis
Taylor & Francis Group

Reversible effect of potassium sorbate on *Balanus amphitrite* larvae. Potential use as antifoulant

Guillermo Blustein[a,b], Miriam Pérez[a,b]*, Mónica García[a], Mirta Stupak[a] and Claudio Cerruti[c]

[a]*CIDEPINT (Centro de Investigación y Desarrollo en Tecnología de Pinturas) CICPBA-CONICET, La Plata, Argentina;* [b]*Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina;* [c]*Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina*

(*Received 3 March 2009; final version received 23 April 2009*)

Marine biofouling constitutes a major worldwide technical and economic problem. International regulations concerning the protection of both the environment and industrial workers have prompted paint manufacturers and end users to look for suitable replacements for traditional antifouling (AF) pigments. For this reason, the potential AF activity of potassium sorbate (KS) on nauplii and cyprids of *Balanus amphitrite* was tested in laboratory and field trials. Larval bioassays demonstrated a marked inhibitory and reversible effect. The values obtained for EC_{50} and LC_{50} were 9.91 mM and 36.73 mM, respectively, and the therapeutic ratio was 3.71, indicating that KS acts *via* a non-toxic mechanism. After 60 days in the sea, a varnish coating incorporating KS showed a substantial decrease in micro- and macrofouling density and diversity. This investigation indicated that KS is a promising AF agent for replacing the traditional toxic compounds.

Keywords: potassium sorbate; antifouling; reversible effect; *Balanus amphitrite* larvae

Introduction

All submerged substrata in marine environments, both natural and artificial, are rapidly colonized by bacteria, plants and animals. This undesirable growth of organisms is called biofouling and starts after any solid surface is immersed in the sea. The colonization of a substratum has been viewed as going through a four-step process, ie biochemical conditioning of the surfaces, bacterial colonization, diatom and protozoan colonization and settlement of invertebrate larvae and algal spores (Wahl 1997; Maki 2002). Marine biofouling modifies the hydrodynamic of ships' hulls and consequently increases fuel consumption, promotes corrosion processes, blocks mollusc and fish culture nets, reduces heat transfer performance on heat exchangers and causes serious problems on cooling systems for power plants (Anon 1952; Muraoka 1968). Another consequence of fouling on ships is the hull transport and/or ballast water spread of invasive species which may lead to the exclusion or displacement of native plant and animal species by competition for food, light and space (Gollasch 2007; Otani et al. 2007).

Generally, fouling development can be prevented by means of antifouling (AF) paints based on copper oxide or tributyltin oxide. For more than 30 years, organotin compounds were the most widely used as AF active ingredients covering >70% of the world's commercial shipping fleet (Yebra et al. 2004). Although organotin containing paints are highly effective, they are also dangerous to the marine environment due to their effects on non-target organisms such as plants, bivalves, fish and marine mammals (Simmonds 1986; Brady 2000). Since the restrictions on the use of TBT in AF applications, several formulations have been developed with possible alternatives to organotin such as Irgarol 1051®, Sea-Nine 211®, chlorothalonil, dichlofluanid, tolylfluanid and zinc pyrithione (Voulvoulis et al. 1999; Konstantinou and Albanis 2004; Bellas 2005). However, these compounds have been found in estuarine and coastal environments, particularly in marinas and harbours at relatively high concentrations (Voulvoulis et al. 2000, 2002; Martínez et al. 2001).

Hence, finding new non-toxic alternatives that exert a specific action on target organisms and that are also biodegradable is urgent (Göransson et al. 2004; Limna Mol et al. 2009). Natural compounds occurring in the marine environment, especially those produced by sessile fouling-free marine organisms, are promising AF agents. The use of materials from marine organisms in AF technologies is based on the concept that these organisms have evolved inhibitory or deterrent mechanisms that protect their own surfaces from being fouled. Many benthic organisms are known to prevent the settlement of fouling organisms because they have

*Corresponding author. Email: biofouling@cidepint.gov.ar

ISSN 0892-7014 print/ISSN 1029-2454 online

DOI: 10.1080/08927010902995572
http://www.informaworld.com

developed a chemical defence against epibionts and predators, and the secondary metabolites of these organisms are assumed to be a source of potential non-toxic AF agents (Clare et al. 1992; Pawlik 1992; Abarzua and Jakubowski 1995; Clare 1996; Armstrong et al. 2000; Rittschof 2001; da Gama et al. 2002; Tsoukatou et al. 2007; Campos De-Paula et al. 2008). These substances incorporated into paints may be less environmentally damaging than the current toxins, and they may have less activity against non-target species (Hellio et al. 2000). However, natural products generally are not available in sufficient quantities to be commercially harvested from marine macroorganisms, and most potent natural product compounds are structurally too complex to be synthesized or cannot be detected because they degrade during collection, storage and extraction (Dobretsov et al. 2006). Moreover, identification and laboratory isolation of natural-origin active compounds may be difficult and sometimes expensive. As yet, the development of AF coatings based on natural products has not been achieved on a commercial scale. In addition to their AF characteristics, products must have properties that make them suitable, eg low toxicity, a broad spectrum of activity against algae and invertebrates and low cost. For this reason, it is important to search for other environmentally friendly alternatives for fouling control. In a previous study (Vetere et al. 1999), it was demonstrated that sodium benzoate, a common food preservative, is a promising AF compound due to the narcotic action of the benzoate anion on *Balanus amphitrite* and *Polydora ligni* larvae. The goal of the present article is to study the behaviour of potassium sorbate (the potassium salt of sorbic acid, see Figure 1), another synthetic compound commonly used as a food preservative in order to evaluate its AF properties. From here on, sorbic acid and potassium sorbate will be named as SA and KS, respectively.

SA is a diunsaturated dicarboxylic acid [(E,E)2,4-hexadienoic acid], which is tasteless and usually found in the fruit (berries) of the mountain ash (*Sorbus aucuparia*). It is used widely as a food preservative to inhibit growth of bacteria, mould and yeasts and its applications expanded rapidly after the issuance of the original patents in 1945 (Gooding 1945). Recently, it was established that SA has a strong antioxidant activity (Korotkova et al. 2005). The FDA regards KS as GRAS (Generally Regarded as Safe).

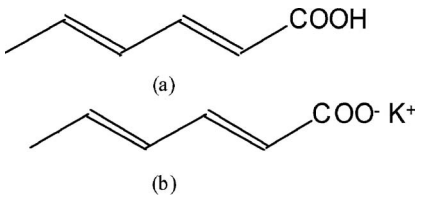

Figure 1. Chemical structures of sorbic acid (a) and KS (b).

SA occurs also in the form of 2-sorboyl-1,3-dimyristin in the fatty deposits of certain aphids (Luck 1993). It reacts with potassium to make KS and with calcium to make calcium sorbate which are also used as antifungal agents.

Several industrial applications of SA have been developed, eg SA improves the characteristics of drying oils, gloss in alkyd type coatings, the milling characteristics of cold rubber and copolymerization processes. It is also used as an intermediate for plasticizers and lubricants. SA is commonly employed as an additive in cosmetic, pharmaceutical and tobacco products.

Besides its frequent use in the food industry, KS has properties such as a corrosion inhibitor (Ein-Eli et al. 2006) and is considered to be an effective copper aqueous corrosion inhibitor (Abelev et al. 2007).

From the point of view of bioactivity as anti-foulant, Ban et al. (2000) reported a potent attachment-inhibiting substance for the blue mussel *Mytilus edulis galloprovincialis* based on a combination of SA and polygodial, a compound extracted from *Tasmannia lanceolata* leaves.

Because SA and KS are non-toxic and biodegradable synthetic chemicals, they are an eco-friendly alternative to be explored as AF agents. In the present article the effect of KS on nauplii and cyprids of *B. amphitrite* Darwin (Cirripedia, Balanidae) in the laboratory and in field trials was studied. Additionally, a further aim was to clarify whether settlement inhibition is a consequence of a toxic or non-toxic effect (Rittschof et al. 1992, 2003).

Material and methods

Experimental solutions

A 100 mM stock solution was made up by dissolving KS powder salt (Sigma-Aldrich) in sterile artificial seawater. The experimental concentrations were obtained by diluting the stock solution. Then, a series of assays were carried out using eight dilutions 100, 50, 25, 20, 12.5, 10, 7.5 and 5 mM.

Fouling organisms

A typical species of fouling organisms at Mar del Plata harbour, Argentine (38°08′17″S, 57°31′18″W), *B. amphitrite* was chosen for the experiments. *B. amphitrite* is a cosmopolitan barnacle species and one of the most successful forms of animal fouling (Koryakova and Korn 1993).

Adult barnacles of *B. amphitrite* were collected from Club de Motonáutica piers and fed daily with a

culture of *Artemia salina* nauplii. In the laboratory, all organisms were 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.

Newly released *B. amphitrite* nauplii I were transferred to a beaker containing sterile artificial seawater and after 1 h they moulted and became nauplii II. Some nauplii II actively swimming towards a light source were used for bioassays and the remainder were maintained with a diet of *Skeletonema costatum*. In these conditions, 30–35% of nauplii metamorphosed into the cyprid stage and they were maintained at 6°C.

Naupliar toxicity test

The effect of each sorbate solution was tested using stage II nauplii of *B. amphitrite*. Larvae were collected from adults 2–4 h before starting bioassays. Toxicity tests were realized by adding 30 nauplii into crystallizing vessels containing 30 ml of either sorbate solutions or filtered seawater as control. The number of swimming nauplii was recorded after exposure to the compounds for 24 h at 22 ± 2°C. The lethal concentration for 50% of nauplii (LC_{50}), is used to identify toxic compounds and it was determined using Sigma Plot. According to Rittschof et al. (1992), non-swimming larvae were regarded as dead and data were expressed with a 99% confidence interval.

Larval swimming inhibition test

Complementary assays to determine larval reaction to solutions during the first 60 min of exposure were observed. Thirty nauplii II were placed into crystallizing vessels containing 30 ml of sorbate solutions at 22 ± 2°C without adding food. The effect of sorbate solutions was estimated by swimming movements (ability/disability to stay in the water column) and phototactic response (positive/negative). Observations were made every 15 min over a period of 1 h and inactive larvae were counted. The concentration of KS causing inhibition of 50% of the swimming nauplii was expressed as IC_{50} and it was determined using Sigma Plot.

Settlement test

Newly metamorphosed cyprids were maintained in filtered seawater for 4 days at 6°C before being used in settlement assays (Rittschof et al. 1992). Twenty cyprids were introduced into crystallizing vessels with 30 ml of each sorbate solution for 24 h at 22 ± 2°C. When larvae did not swim or close their valves or if their appendages were extended and they did not respond when touched with a metal probe they were counted as inactive cyprids. The percentages of swimming, inactive and settled individuals were determined by counting under a dissecting microscope, and the results are expressed as a proportion of the total number of larvae in the vessel. Settlement data for EC_{50} (concentrations of sorbate solutions causing settlement inhibition in 50% of experimental organisms) were obtained by normalizing settlement values to control prior to analysis. EC_{50} was determined using Sigma Plot.

Reversibility test

In the laboratory, toxic compounds are not differentiated from narcotic compounds and, for this reason, larvae were placed in seawater to determine if they recovered. In order to investigate if KS acts by a temporary or a permanent mechanism, reversibility assays were carried out. After 24 h exposure to KS, nauplii and cyprids were transferred to clean seawater to determine whether the effects observed were reversible.

Measurements of KS release from gels

In the laboratory, the release of the KS from the Phytagel™ matrix into artificial seawater without stirring was investigated.

The procedure for immobilization of KS into the gel was made by dissolution of 7.2 g Phytagel™ (Sigma Chemical) in 180 ml distilled water in a microwave oven. After cooling at 50°C, 20 ml of 2 M solution of KS was incorporated with vigorous stirring. Then, mixture was poured into six sterile hermetic plastic containers which were kept in the dark for 24 h at 4°C for stabilization. Each recipient was filled with 80 ml sterile artificial seawater in order to obtain sorbate diffusion release for 2, 4, 6, 12, 18 and 24 h. For analytical measurements, a series of control gels were prepared in a similar manner except for the addition of 20 ml of distilled water instead of KS. All determinations were conducted with three replicates.

The quantities of sorbate in the wash-out solutions were determined directly by high performance liquid chromatography (HPLC). The chromatographic analyses were carried out in a Shimadzu high-performance liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with two LC-10AS pumps, a CTO-10A column oven, a 7725 Rheodyne manual injector (Cotati, CA, USA) and a model SPD-10A variable-wavelength UV–vis detector using an SCL-10A system controller module and a C-R7A chromatopac integrator.

The quantitative HPLC separations were performed at a temperature of 30°C (controlled by the oven) on a LiChrospher 100 RP-18, 125 mm × 4 mm, particle size 5 μm, (Merck) reversed-phase column. The mobile phase was a 0.5 g l^{-1} sodium acetate solution (pH 4.3). The flow rate was 1 ml min^{-1} and the absorbance detector was set at 238 nm.

AF activity of KS in the field

In order to determine the AF effect of KS, the compound was mixed into a non-toxic varnish and immersed in Mar del Plata harbour.

The varnish utilized to immobilize the active AF compound was prepared by dispersing KS powder salt (Sigma-Aldrich) into ALKIPOL 434-50® Resin (Polidur San Luis S.A.I.C.) employing a ball mill for 24 h (see Table 1). Once the varnish was prepared, it was filtered through a Lycra® grid in order to eliminate environmental dust and/or bigger particles. The varnish was applied to sandblasted acrylic panels (128 cm^2) by paint brush to reach a dry-film thickness of 100 μm ± 5 μm. Painted panels were kept indoors for 7 days before testing. A series of control panels were painted only with resin (without sorbate). Also, a series of unpainted acrylic tiles were used to establish fouling community development. Experiments were performed with three replicates. After exposure for 60 days, the settlement of fouling organisms was measured as percentage cover on each panel using a dot-grid estimate method (Foster et al. 1991).

Data analysis

Average and standard errors were calculated from the results of laboratory assays. All experiments were performed as five replicates and controls. As the data obtained were normally distributed (Shapiro–Wilks normality test), they were analysed by ANOVA and Tukey pair-wise comparison tests. The level of significance was set at $p < 0.05$.

The therapeutic ratio (TR) was determined by dividing the concentration for 50% mortality (LC_{50}) by the concentration for 50% settlement inhibition (EC_{50}) (Vitalina et al. 1991; Rittschof et al. 1994).

Table 1. Varnish composition by % v/v.

Resin (Medium oil alkyd)	AF agent (Potassium sorbate)	Solvent (White spirit)
31.9	48.6	19.5

Results

A wide range of sorbate concentration was tested to determine its ability to repel *B. amphitrite* larvae. Naupliar toxicity tests (LC_{50}), larval swimming inhibition (IC_{50}) and cyprids settlement assays (EC_{50}) are presented in Table 2.

Naupliar toxicity test

Laboratory tests conducted using nauplii II of *B.amphitrite* after 24 h incubation in KS solutions are presented in Figure 2. The results indicate that nauplii were affected by exposure to solutions of increasing KS concentration in seawater. The behavioural response of nauplii was similar in all cases, ie larvae lost their phototactic response, became immediately quiescent, immobilized their appendages and stopped their swimming movements.

As seen in Figure 2, significant differences between treatments and control were observed in solutions >10 mM; above this concentration solutions were effective in inhibiting naupliar activity ($p < 0.01$). The lowest concentration to immobilize 50% of nauplii was

Table 2. Calculated concentrations of KS from different tests on *B. amphitrite* larvae.

	Toxicity (LC_{50})	Swimming inhibition (IC_{50})	Cyprid settlement (EC_{50})
Nauplii	36.73 mM	10.34 mM	
Cyprids	>50.00 mM	11.61 mM	9.91 mM

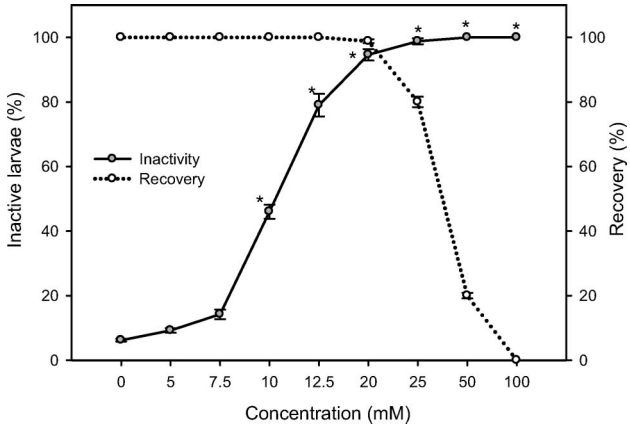

Figure 2. Inhibition of swimming of stage II nauplii of *B. amphitrite* exposed for 24 h to different concentrations of KS and recovery in clean seawater. Bars = mean ± SE; *significant difference from control.

determined at 10.34 mM, but toxicity was established at 36.73 mM (LC_{50}).

Larval swimming inhibition test

Larval response registered during the first 60 min of exposure to KS solutions was similar to that observed for 24 h, ie swimming inhibition of nauplii was determined at and above 10.0 mM ($p < 0.05$) (Figure 3). It is important to note that at concentrations of 12.5 and 20.0 mM this effect became faster and massive, affecting 80 and 90% of the larval population, respectively. Experiments showed that KS acted immediately on nauplii and its effect was maintained over time (at least 24 h).

Cyprid settlement test

The antisettlement activity of KS was examined by exposure of cyprids for 24 h. The onset time for cyprid immobility to occur was 2–3 min. In most of the cases, cyprid behaviour was immediately affected by exposure to the compound, swimming cyprids responded with a rapid immobilization and of closing their valves.

One-way ANOVA detected significant differences in the settlement of cyprid larvae with regard to the concentration used ($p < 0.05$) (Figure 4). The KS threshold molarity which inhibited larval settlement was 10 mM and the EC_{50} value obtained was 9.91 mM.

Reversibility test

After 24 h exposition, nauplii and cyprids were transferred to fresh artificial seawater and the percentage recovery was estimated. In the range 5–20 mM, all nauplii recovered swimming movements but at higher concentrations the recovery percentage was lower (Figure 2). Except for 100 mM, cyprids recovered from the effects of KS within few minutes and after a further 24–48 h they could complete metamorphosis and settlement.

Release of KS from gels

Figure 5 illustrates the release of KS embedded in the gel. The compound was quickly leached out from the inert matrix into non-stirred seawater. Fifty per cent of the original load of sorbate in the gel was lost within the first 6 h of exposure and the amount of KS in the wash-out solution increased from 50 to ~80% within

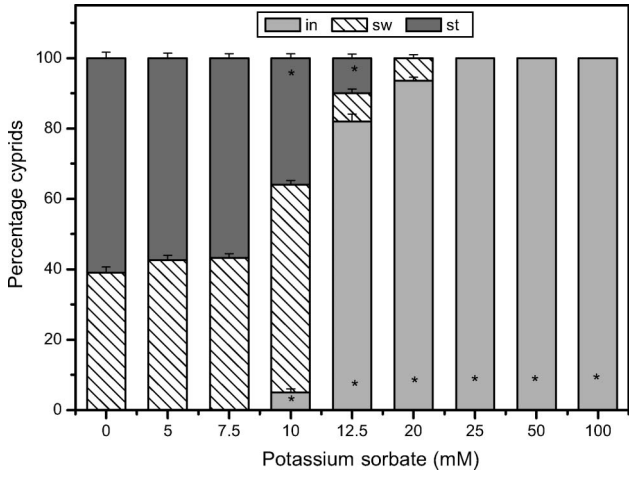

Figure 4. Effect of KS solutions on cyprids. Results are expressed as percentage of inactive larvae (in), swimmers (sw) and settlers (st). Bars = mean ± SE; *significant difference from control.

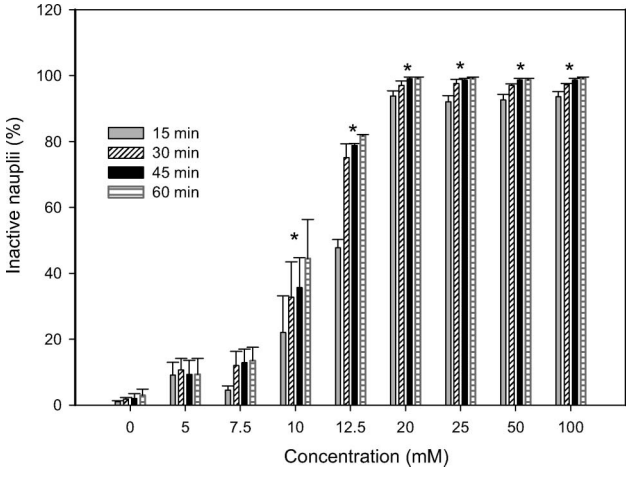

Figure 3. Effect of KS solutions on nauplii II during an exposure period of 60 min. Bars = mean ± SE; *significant difference from control.

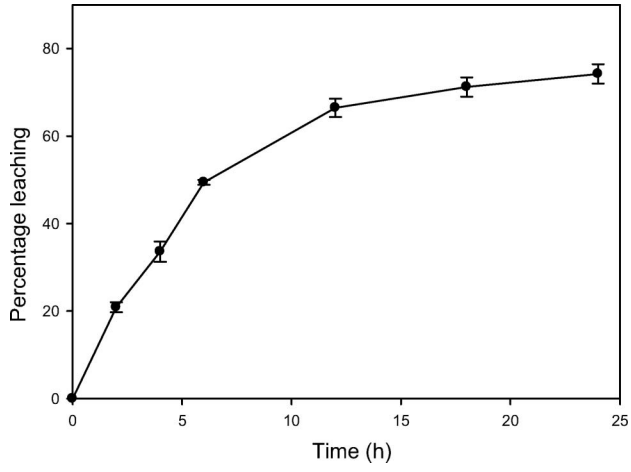

Figure 5. Percentage release of KS from gels as a function of exposure time in non-stirred seawater. Bars = mean ± SE.

the next 24 h. The fast leakage of sorbate from the gels in a short period of time and static conditions is a limiting factor for evaluating the AF properties of sorbate in the field trials. For this reason, KS was included into a varnish.

AF activity of KS in the field

After 60 days in the sea, the percentage cover of biofouling on panels was estimated. KS incorporated into a varnish coating showed a substantial decrease in the density and diversity of micro- and macrofouling. Significative differences were registered in the settlement of diatoms (*Achnanthes* sp., *Nitzschia* spp., *Synedra* sp., *Lycmophora* sp.) and some macrofoulers (*Enteromorpha intestinalis, Ectocarpus* sp., *B. amphitrite, Bugula* spp., *Ciona intestinalis, Botryllus* spp.) between KS painted panels and control panels ($p < 0.05$).

Discussion

This study is focussed on the use of KS as an antifoulant and its effects on the early developmental stages of *B. amphitrite* were evaluated. In the laboratory, exposition of nauplii and cyprids to solutions of this compound demonstrated a marked inhibitory and reversible effect.

KS has a behaviour-altering effect on nauplii and cyprid stages. Effective concentrations for the compound as antifoulant were those at and above 20 mM in which settlement was completely inhibited. Larval bioassays provided information about the mode of action of KS. This effect of KS is anaesthetic (or narcotic) for a broad range of concentrations.

The TR, LC_{50}/EC_{50} is a way of expressing the effectiveness of the compound in relation to its toxicity. It is calculated to determine whether settlement inhibition is due to toxic action or some other mechanism (Vitalina et al. 1991; Rittschof et al. 1994). From the perspective of potency for use in an AF coating, the desired target ratio should be much greater than 1.0 (Rittschof et al. 2003). KS is of particular interest because it shows good antisettlement activity at concentrations that were not acutely toxic to barnacle larvae, with a TR = 3.71, thus satisfying the above-mentioned criterion. Additionally, the effect can be reversed by removal of the larvae from the KS solution, demonstrating that KS acts *via* a non-toxic mechanism, which has not been elucidated hitherto.

However, growth inhibition of microorganisms by KS has been known from many years and involves diverse mechanisms, for instance, cytoplasm acidification during weak-acid stress in both yeasts and moulds (Krebs et al. 1983; Plumridge et al. 2004) and inhibition of enzyme activity and amino acid transport (Freese et al. 1973; Krebs et al. 1983). Also, it has been established that accumulation of the anion fraction of dissociated preservatives causes oxidative stress and increases turgor pressure within the cell (Piper 1999). Concerning the toxicological aspects of KS and SA, several *in vitro* and *in vivo* studies have shown that both compounds are non-mutagenic and non-clastogenic (Winkler et al. 2006) and that they are rapidly metabolized because they follow the same pathways as other fatty acids (Walker 1990). Besides, neither genotoxicity nor carcinogenicity has been detected in cultured human cells exposed to KS (Mpountoukas et al. 2008). Furthermore, acute fish toxicity is very low and also very little toxicity to plants has been shown (Luck 1993).

Some microorganisms are capable of degrading and metabolizing SA, usually by means of enzymatic decarboxylation reaction (Casas et al. 2004; Pinches and Apps 2007; Plumridge et al. 2008). Particularly, degradation of SA by strictly anaerobic Gram-negative sulfate-reducing and fermenting bacteria has been reported (Schnell et al. 1991). On the other hand, laboratory experiments demonstrated that degradation products of KS have an inhibitory action on the growth of *Staphylococcus aureus* (Campos et al. 2000).

The inhibition of the organism settlement process could be achieved by breaking one or more of the steps implicated in biofouling development (Hellio et al. 2001). Studies have shown that bacterial biofilms can play an important role in mediating settlement and metamorphosis of larvae, eg they can enhance or inhibit larval and algal spore attachment (Rodríguez et al. 1993; Lau and Qian 1997; O'Connor and Richardson 1998; Huang and Hadfield 2003; Qian et al. 2003). Microbial cells in biofilms are enmeshed in a matrix of extracellular polymers that are mainly composed of high-molecular weight polysaccharides (Decho 2000). A broad range of marine invertebrate larvae utilizes biofilms as indicators of substratum suitability for prospective settlement (Dobretsov et al. 2006). The physical and dynamic properties of biofilms, and the biotic composition and accumulation of chemical compounds provide a discriminative mechanism in shaping biofouling communities (Hodgson 1990; James and Underwood 1994; Qian 1999; Qian et al. 2000).

It is well established that KS has antimicrobial properties, and for this reason it is hypothesized that it could affect the progress of biofilm formation. Although in field trials KS demonstrated an inhibitory effect on diatoms, the total components of biofilms have not yet been explored. This subject will be the focus of future work.

It was not possible to study the AF effect of KS immobilized into Phytagel discs in the sea; the KS was released from the gels in a few hours and caused a sudden drop in the concentration in gel discs and/or in

the disc surfaces. Consequently, KS was incorporated into a varnish to be evaluated in the sea. From preliminary short-term assays, a substantial decrease in the density and diversity of micro- and macrofouling was observed. Therefore, KS holds promise as an environmentally friendly antifoulant.

Final remarks

Further research is needed in two major directions, *viz* to determine the antimicrobial activity of KS against the marine bacteria involved in the formation of the microlayer and to evaluate the performance and effectiveness of KS included in potential paint matrices under natural conditions. KS is a cheap and biodegradable commercial chemical which has marked antisettlement activity and a reversible effect. It is also non-lethal and has very low toxicity. In view of these facts, it was concluded that KS is a suitable eco-friendly alternative for use as an AF agent.

Acknowledgements

The authors thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CIC) and Universidad Nacional de La Plata (UNLP) for their economic support. They also thank the Club de Motonáutica of Mar del Plata for the permission to use their marine testing site and the anonymous reviewers who provided useful comments on the manuscript.

References

Abarzua S, Jakubowski S. 1995. Biotechnological investigation for the prevention of biofouling. I. Biological and biochemical principles for the prevention of biofouling. Mar Ecol Prog Ser 123:301–312.

Abelev E, Starosvetsky D, Ein-Eli Y. 2007. Potassium sorbate. A new aqueous copper corrosion inhibitor. Electrochemical and spectroscopic studies. Electrochim Acta 52:1975–1982.

Anon. 1952. Marine fouling and its prevention. Annapolis (MD): US Naval Institute.

Armstrong E, Boyd KG, Burgess JG. 2000. Prevention of marine biofouling using natural compounds from marine organisms. Biotech Annu Rev 6:221–241.

Ban T, Singh IP, Etoh H. 2000. Polygodial, a potent attachment-inhibiting substance for the blue mussel, *Mytilus edulis galloprovincialis* from *Tasmannia lanceolata*. Biosci Biotechnol Biochem 64:2699–2701.

Bellas J. 2005. Toxicity assessment of the antifouling compound zinc pyrithione using early developmental stages of the ascidian *Ciona intestinalis*. Biofouling 21:289–296.

Brady RF, Jr. 2000. No more tin. What now for fouling control? J Prot Coat Lin (JPCL) PCE, June: 42–46.

Campos CA, Alzamora SM, Gerschenson LN. 2000. Inhibitory action of potassium sorbate degradation products against *Staphylococcus aureus* growth in laboratory media. Int J Food Microbiol 54:117–122.

Campos De-Paula J, Bueno L, Cavalcanti DN, Yoneshigue-Valentin Y, Teixeira VL. 2008. Diterpenes from the brown alga *Dictyota crenulata*. Molecules 13:1253–1262.

Casas E, Ancos B, Valderrama MJ, Cano P, Peinado JM. 2004. Pentadiene production from potassium sorbate by osmotolerant yeasts. Int J Food Microbiol 94:93–96.

Clare AS. 1996. Marine natural product antifoulants: status and potential. Biofouling 9:211–229.

Clare AS, Rittschof D, Gerhart DJ, Maki JS. 1992. Molecular approaches to nontoxic antifouling. Invert Reprod Dev 22:67–76.

da Gama B, Pereira R, Carvalho A, Coutinho R, Yoneshigue-Valentin Y. 2002. The effects of seaweed secondary metabolites on biofouling. Biofouling 18: 13–20.

Decho AW. 2000. Microbial biofilms in intertidal systems: an overview. Continent Shelf Res 20:1257–1273.

Dobretsov S, Dahms HU, Qian P-Y. 2006. Inhibition of biofouling by marine microorganisms and their metabolites. Biofouling 22:43–54.

Ein-Eli Y, Abelev E, Starosvetsky D. 2006. Food preservatives serving as nonselective metal and alloy corrosion inhibitors. Electrochem Sol Stat Lett 9:B5–B7.

Foster MS, Harrold C, Hardin D. 1991. Points *versus* photo quadrat estimates of the cover of sessile marine organisms. J Exp Mar Biol Ecol 146:193–203.

Freese E, Sheu CW, Galliers E. 1973. Function of lipophilic acids as antimicrobial food additives. Nature 241:321–325.

Gollasch S. 2007. International collaboration on marine bioinvasions. The ICES response. Mar Poll Bull 55:353–359.

Gooding CM. 1945. Process of inhibiting growth of molds. US Patent 2,379,294.

Göransson U, Sjögren M, Svangärd E, Claeson P, Bohlin L. 2004. Reversible antifouling effect of the cyclotide cycloviolacin O_2 against barnacles. J Nat Prod 67:1287–1290.

Hellio C, Bremer G, Pons AM, Le Gal Y, Bourgougnon N. 2000. Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. Appl Microbiol Biotechnol 54:543–549.

Hellio C, De La Broise D, Dufossé L, Le Gal Y, Bourgougnon N. 2001. Inhibition of marine bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints. Mar Environ Res 52:231–247.

Hodgson G. 1990. Sediment and the settlement of larvae of the reef coral *Pocillopora damicornis*. Coral Reefs 9:41–43.

Huang S, Hadfield M. 2003. Composition and density of bacterial biofilms determine larval settlement of the polychaete *Hydroides elegans*. Mar Ecol Prog Ser 260:161–172.

James RJ, Underwood AJ. 1994. Influence of colour of substratum and recruitment of spirorbid tubeworms to different types of intertidal boulders. J Exp Mar Biol Ecol 181:105–115.

Konstantinou IK, Albanis TA. 2004. Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. Environ Int 30:235–248.

Korotkova EI, Avramchik OA, Angelov TM, Karbainov YA. 2005. Investigation of antioxidant activity and lipophilicity parameters of some preservatives. Electrochim Acta 51:324–332.

Koryakova M, Korn O. 1993. Using barnacle larvae for evaluation of the toxicity of antifouling paints compounds. Russ J Mar Biol 19:212–216.

Krebs HA, Wiggins D, Stubs M, Sols A, Bedoya F. 1983. Studies on the mechanism of the antifungal action of benzoate. Biochem J 214:657–663.

Lau SCK, Qian PY. 1997. Phlorotannins and related compounds as larval settlement inhibitors of the tube-building polychaete *Hydroides elegans*. Mar Ecol Prog Ser 159:219–227.

Limna Mol VP, Raveendran TV, Parameswaran PS. 2009. Antifouling activity exhibited by secondary metabolites of the marine sponge, *Haliclona exigua* (Kirkpatrick). Int Biodeterior Biodegr 63:67–72.

Luck E. 1993. Sorbic acid. In: Elvers B, Hawkins S, Russey W, Schulz G, editors. Ullmann's encyclopedia of industrial chemistry. A 24. New York: VCH Publishers. p. 507–513.

Maki J. 2002. Biofouling in the marine environment. In: Bitton H, editor. Encyclopedia of environmental microbiology. New York: Wiley & Sons. p. 610–619.

Martínez K, Ferrer I, Hernando MD, Fernández-Alba AR, Marce RM, Borrul F. 2001. Occurrence of antifouling biocides in the Spanish Mediterranean marine environment. Environ Technol 22:543–553.

Mpountoukas P, Vantarakis A, Sivridis E, Lialiaris T. 2008. Cytogenetic study in cultured human lymphocytes treated with three commonly used preservatives. Food Chem Toxicol 46:2390–2393.

Muraoka JS. 1968. Effects of marine organisms. Machine Des 18:184–187.

O'Connor NJ, Richardson DL. 1998. Attachment of barnacle (*Balanus amphitrite* Darwin) larvae: responses to bacterial films and extracellular materials. J Exp Mar Biol Ecol 226:115–129.

Otani M, Oumi T, Uwai S, Hanyuda T, Prabowo RE, Yamaguchi T, Kawai H. 2007. Occurrence and diversity of barnacles on international ships visiting Osaka Bay, Japan, and the risk of their introduction. Biofouling 23:277–286.

Pawlik JR. 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr Mar Biol Annu Rev 30:273–335.

Pinches SE, Apps P. 2007. Production in food of 1,3-pentadiene and styrene by *Trichoderma* species. Int J Food Microbiol 116:182–185.

Piper PW. 1999. Yeast superoxide dismutase mutants reveal a pro-oxidant action of weak organic acid food preservatives. Free Radic Biol Med 27:1219–1227.

Plumridge A, Stratford M, Lowe KC, Archer DB. 2008. The weak-acid preservative sorbic acid is decarboxylated and detoxified by a phenylacrylic acid decarboxylase, PadA1, in the spoilage mold *Aspergillus niger*. Appl Environ Microbiol 74:550–552.

Plumridge A, Hesse SJ, Watson AJ, Lowe KC, Stratford M, Archer DB. 2004. The weak acid preservative, sorbic acid, inhibits conidial germination and mycelial growth of *Aspergillus niger* through intracellular acidification. Appl Environ Microbiol 70:3506–3511.

Qian PY. 1999. Larval settlement of polychaetes. Hydrobiologia 402:239–253.

Qian PY, Rittschof D, Sreedhar B. 2000. Macrofouling in unidirectional flow: miniature pipes as experimental models for studying the interaction of flow and surface characteristics on the attachment of barnacle, bryozoan and polychaete larvae. Mar Ecol Prog Ser 207:109–121.

Qian PY, Thiyagarajan V, Lau SCK, Cheung S. 2003. Relationship between microbial community and the attachment of acorn barnacle *Balanus amphitrite* Darwin. Aquat Microb Ecol 33:225–237.

Rittschof D. 2001. Natural product antifoulants and coatings development. In: McClintock B, Baker B, editors. Marine chemical ecology. Boca Raton (FL): CRC Press. p. 543–566.

Rittschof D, Ali C-H, Kok L-M, Teo SL-M. 2003. Pharmaceuticals as antifoulants: concept and principles. Biofouling 19:207–212.

Rittschof D, Clare AS, Gerhart DJ, Mary A, Bonaventura J. 1992. Barnacle *in vitro* assays for biologically active substances: toxicity and settlement inhibition assays using mass cultured *Balanus amphitrite* Darwin. Biofouling 6:115–122.

Rittschof D, Sasikumar N, Murlless D, Clare AS, Gerhart CD, Bonaventura J. 1994. Mixture interactions of lactones and furans and a commercial biocide: toxicity and antibarnacle settlement activity. In: Thompson M-F, Nagabhushanam R, Sarojini R, Fingerman M, editors. Recent developments in biofouling control. Rotterdam: Balkema AA. p. 269–274.

Rodríguez SR, Ojeda FP, Inestrosa NC. 1993. Settlement of benthic marine invertebrates. Mar Ecol Prog Ser 97:193–207.

Schnell S, Wondrak C, Wahl G, Schink B. 1991. Anaerobic degradation of sorbic acid by sulfate-reducing and fermenting bacteria: pentanone-2 and isopentanone-2 as by products. Biodegradation 2:33–41.

Simmonds M. 1986. The case against tributyltin. Oryx 20:217–220.

Tsoukatou M, Maréchal JP, Hellio C, Novakovic I, Srdan T, Sladic D, Gasic M, Clare A, Vagias C, Roussis V. 2007. Evaluation of the activity of the sponge metabolites avarol and avarone and their synthetic derivatives against fouling micro- and macroorganisms. Molecules 12:1022–1034.

Vetere V, Pérez M, García M, Deyá M, Stupak M, del Amo B. 1999. A non-toxic antifouling compound for marine paints. Surf Coat Intern (JOCCA) 82:586–589.

Vitalina S, Avelin S, Rittschof D, Sarojini R, Nagabhushanam R. 1991. Compounds from octocorals that inhibit barnacle settlement; isolation and biological potency. In: Thompson M-F, Sarojini R, Nagabhushanam R, editors. Bioactive compounds from marine organisms. Rotterdam: Balkema AA. p. 331–339.

Voulvoulis N, Scrimshaw MD, Lester JN. 1999. Alternative antifouling biocides. Appl Organomet Chem 13:135–143.

Voulvoulis N, Scrimshaw MD, Lester JN. 2000. Occurrence of four biocides utilized in antifouling paints, as alternatives to organotin compounds, in waters and sediments of a commercial estuary in the UK. Mar Pollut Bull 40:938–946.

Voulvoulis N, Scrimshaw MD, Lester JN. 2002. Partitioning of selected antifouling biocides in the aquatic environment. Mar Environ Res 53:1–16.

Wahl M. 1997. Living attached: aufwuchs, fouling, epibiosis. In: Nagabhushanam R, Thompson M, editors. Fouling organisms of the Indian Ocean: biology and control technology. New Delhi: Oxford & IBH Publishing Company. p. 31–84.

Walker R. 1990. Toxicology of sorbic acid and sorbates. Food Addit Contam 7:671–676.

Winkler C, Frick B, Schroecksnadel K, Schennach H, Fuchs D. 2006. Food preservatives sodium sulfite and sorbic acid suppress mitogen-stimulated peripheral blood mononuclear cells. Food Chem Toxicol 44:2003–2007.

Yebra DM, Kiil S, Dam-Johansen K. 2004. Antifouling technology – past, present and future steps towards efficient and environmentally friendly antifouling coatings. Prog Org Coat 50:75–104.