Biotechnological investigation for the prevention of biofouling. I. Biological and biochemical principles for the prevention of biofouling

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ABSTRACT: The most important biological and biochemical methods with potential for the prevention of biofouling are described. Among these methods, the isolation of biogenic agents produced by several species of micro- and macroalgae and marine invertebrates with antibacterial, antialgal, antiprotozoan and antimacrofouling properties may be the most promising and effective method for the prevention of biofouling. The isolated substances with the most potent antifoulant activity are fatty acids, terpenes, terpenoids, lipoproteins, glycolipids, phenols, lactones, peptides and steroids. The advantage of the utilization of micro- and macroalgae for the isolation of biogenic agents is that algae can be cultivated in a short time in mass culture, independent of season. Furthermore, they can be manipulated to a large extent in the direction of the 'production of biogenic agents'. However, the cultivation of micro- and macroalgae is very expensive. Marine invertebrates must be collected in certain seasons. This collection of marine invertebrates could lead to an uncontrolled exploitation of marine organisms and to a change in the balance of marine ecosystems. Therefore, determination of the chemical structure and the subsequent synthesis of the determined biogenic agents is necessary if marine invertebrates are to be used as producers of biogenic agents. Antifouling systems must be both environmentally safe and effective for at least 3 yr when formulated as antifouling paints. There have been a few attempts at this, but no applicable successes have been reported to date.

KEY WORDS: Antifouling · Biofouling · Growth inhibition · Marine bioactive agents · Macrofoulers · Microfoulers · Settlement

INTRODUCTION

What is biofouling?

Biofouling is one of the most important problems currently facing marine technology. In the marine environment any solid surface will become fouled. Materials submerged in seawater experience a series of discrete physical, chemical and biological events which results in the formation of a complex layer of attached organisms known as biofouling.

Loeb & Neihof (1975), Baier (1984) and Lewin (1984) have shown that the first event is the accumulation of an organic 'conditioning' film consisting of chemical compounds (mostly protein, proteoglycans and polysaccharides) making the surface wettable (Dexter 1978). This process occurs in the first minutes of the biological settlement (Fig. 1). After approximately 1 to 2 h, the colonization of bacteria involving 2 distinct phases, a reversible approach phase ('adsorption') and a nonreversible attachment phase ('adhesion'), occurs (Marshall 1980, Wahl 1989) (Fig. 1). The first, reversible adsorption, is an instantaneous attraction which holds bacteria near the surface. The bacterial adsorption is essentially governed by physical forces: Brownian motion, electrostatic interaction, gravity, and van-der-Waal forces (Fletcher & Loeb 1979, Walt et al. 1985). The phenomenon is termed reversible because the organisms can easily be removed before substantial contact o' cell surface has been made. The second, irreversible attachment phase can be made by bacteria that produce extracellular bridging polymer (e.g. poly-
saccharide fibrils consisting mostly of glucose and fructose. It is known that these polysaccharide fibrils (slimes) are anchored to their chemical counterparts in the macromolecular film by lectins or divalent cations (Ca$^{2+}$, Mg$^{2+}$) (Costerton et al. 1978). With the establishment of covalent bonds between the bacterial glyocalix and the macromolecular film the adsorption phase blends into the adhesion phase (Wahl 1989). The growing bacterial lawn, composed of dead and living cells and their secreted ‘slime’, together with the macromolecular film, constitutes the so-called primary film (slime film) (Wahl 1989).

Diatoms, spores of macroalgae and protozoa appear after the development of the primary film (Fig. 1) with a clear quantitative dominance of the diatoms (Marshall et al. 1971, Caron & Sieburth 1981, Cuba & Blake 1983, Zahuranec 1991). Benthic diatoms are attached by mucus secretion (Cooksey et al. 1984, Ferreira & Seeliger 1985) and may densely cover wide substratum areas. While in the majority of observed events diatom colonization was always preceded by bacterial attachment (Little 1984), there may be exceptions (Sieburth & Tootle 1981, Maki et al. 1988). Attachment of spores of macroalgae is realized by species such as Enteromorpha intestinalis and Ullothrix zonata; protozoan colonizers belong mostly to the sessile or semisessile forms (e.g. Ciliata) or are mobile predators of microorganisms, not being considered to be true epibionts.

Bacteria and diatoms represent the primary colonizers and spores of macroalgae and protozoa constitute the secondary colonizers in the process of microfouling (Fig. 1). A clear separation between microfouling and macrofouling is impossible because the spores of macroalgae belong to the macrofouling organisms (Von Oertzen et al. 1989), therefore there is an overlapping between micro- and macrofouling (Fig. 1). Larvae of macrofoulers (sessile marine organisms such as tunicates, coelenterates, bryozoans, barnacles, mussels, polychaetes), which are called tertiary colonizers, then attach to the microfouling film (Fig. 1) (Takazawa et al. 1992). Larvae of macrofoulers prefer to settle on surfaces coated with microbial and algal films (Colwell 1983).

According to micro- and macrofouling processes the following overlapping time sequence is observed: bacteria appear after approximately 1 to 2 h, diatoms after 24 h, spores of macroalgae and protozoa after 1 wk and larvae of macrofoulers after 2 to 3 wk (Von Oertzen et al. 1989). It seems that all micro- and macrofoulers produce adhesive substances necessary for their attachment to solid surfaces such as the hull of a ship (Cooksey et al. 1984). In the literature there are contradictory opinions on the question whether this sequence of events constitutes a real ecological succession or not (Little 1984).

The settlement of micro- and macrofoulers on the hulls of ships must be prevented for both economic and ecological reasons. The bacterial slime films and the large numbers of barnacles, mussels and tunicates which accumulate on ships increase drag forces and surface corrosion, thereby causing additional fuel, CO$_2$ emissions and maintenance costs (Gitlitz 1981).

**Effect of the utilization of antifouling paints**

In the efforts to avoid marine biofouling, antifouling paints are used, mostly with copper and tri-n-
butyltin (TBT) as very effective active agents (Willemsen & Ferrar 1993). The antifouling paints prevent biofouling by releasing effective biocides at a constant rate. Since the early 1970s, triaryltin and trialkyltin compounds have been increasingly used in antifouling paints because of their excellent ability to prevent marine organisms from becoming encrusted on ship bottoms and culturing nets (Suzuki et al. 1992).

TBT is used as a biocide in coatings in 3 different ways: in free association paints (biocide dispersed in a resinous matrix), in ablative paints (biocide is bonded in a less permeable matrix that gradually flakes off) and in self-polishing copolymers (SPC) (biocide is chemically bonded). Self-polishing organotin copolymer formulations have the best release rate characteristics of currently available antifouling paints and are capable of maintaining vessels free from macroscopic biofouling for periods of up to 5 yr (Christie & Dalley 1987). In use as an antifouling additive the use of marine paints containing the broad-spectrum poison TBT grew to an estimated 136 000 kg yr⁻¹ by the late 1980s (Uhler et al. 1993). The worldwide application of TBT-based paints has caused a growing pollution of the environment and foods on a worldwide scale (Suzuki et al. 1992). TBT harms many forms of marine life other than fouling organisms, including economically important species like oysters. Thus, shell deformation of the Pacific oyster Crassostrea gigas, and little or no natural oyster larval settlement on hard substrates, suggesting toxic effects in early life stages, were observed in Arcachon Bay, France. More extreme deformations occur in the common dogwhelk Nucella lapillus, a species of thick-shelled snail found around the southwest peninsula of England. The occurrence of imposex (the development of male characteristics, notably a penis and a sperm duct, on females) was shown in centres of boating and shipping activities, which is a sign of decline (Oehlmann et al. 1993). Fewer females occur than would be expected, and juveniles and deposited egg capsules are scarce or absent, indicating a low reproductive capacity.

Due to risk in application of organotin antifouling coatings they have come under increasing governmental regulation in the United States and many western European countries (Price et al. 1992). This has initiated further development of TBT-free antifouling paints, containing herbicides, antibiotica, copper salts and other organic additives like amino-, azine- and thio-derivatives (Golchert 1993, Peterson et al. 1993). These TBT-free antifouling coatings are capable of maintaining ships free from biofouling for about 3 yr but none of these additives can compete with organotin containing SPCs (Golchert 1993).

**Alternative methods for the prevention of biofouling**

Due to present and expected future restrictive regulation on the use of TBT (Dalley 1987) and probably other polluting antifouling compounds there is a growing need for other methods of the prevention of biofouling. Several physical/mechanical, physical/chemical and biological/biochemical principles for the prevention of biofouling were used in the last 30 yr (Gerencser et al. 1962, Schulz & Subklef 1964, Kuhl & Neumann 1969, Loeb & Neihof 1975, Characklis 1981, Dhar et al. 1981, Branscomb & Rittschof 1984, Fletcher & Baier 1984, Humphries et al. 1986).

It is the aim of the present work to describe and evaluate the most important biological/biochemical methods used for the prevention of settlement and to discuss the possibility of using biogenic agents produced by several marine organisms with antibacterial, antialgal, antiprotozoan and antimacrofouling properties for the prevention of biofouling.

**BIOLOGICAL AND BIOCHEMICAL METHODS FOR THE PREVENTION OF BIOFOULING**

**Dissolution of adhesive substances by several enzymes**

All biofouling organisms achieve their attachment by using adhesive substances, the chemical structure of which is quite similar for bacteria, diatoms, spores of macroalgae and macrofoulers (acid polysaccharides and glycoproteins) (Fletcher & Floodgate 1973, Haug 1976, Humphrey et al. 1979, Percival 1979, Daniel et al. 1987, Wigglesworth-Cooksey & Cooksey 1992). It is assumed that a dissolution of these substances would reduce the long-term attachment to solid surfaces (Christie et al. 1970, Dempsey 1981, Evans 1981, Cinti et al. 1987). Certain enzymes, like trypsin, chymotrypsin, pronase and α-amylase, are active in the weakening action on adhesion in the bacterium Vibros proteolytica and in zoospores of the green macroalga Enteromorpha intestinalis (Christie et al. 1970, Paul & Jeffrey 1985). Short-term exposure to the enzymes actinidin and pepsin can be used to separate the stalked epiphytic diatoms Synedra tabulata and Licmophora species from their brown or green algal hosts (Boot 1981). A variety of hydrolytic enzymes were tested for effects on barnacle settlement on solid surfaces (Rittschof et al. 1991). The majority of enzymes tested (cellulase, chitinase, collagenase, trypsin, chymotrypsin, carboxypeptidase A, B, Y) had little effect on settlement. But protease XI significantly inhibited the settlement of barnacles on polystyrene and glass surfaces, while papain had an inhibitory
effect on polystyrene surfaces only. The biotechnological production of these enzymes in large quantities is possible, but relatively expensive. Furthermore, the enzymes are not permanently stable and different enzymes are necessary to split the various adhesive substances. Therefore, the method of dissolution of adhesive substances is applied only partly in practice.

**Intervention in the metabolism of fouling organisms**

A well-balanced supply of calcium is necessary for a successful adhesion of bacteria, diatoms and spores of macroalgae (Marshall et al. 1971, Grant et al. 1973, Haug 1976, Fletcher 1979, Cooksey & Cooksey 1980, Cooksey et al. 1981, Turakhia & Characklis 1989). The process of synthesis and secretion of adhesive substances leading to motility and adhesion in diatoms can be prevented by uncouplers of energy metabolism (CCCP, carbonyl cyanide 3-chlorophenyl hydrazone), protein synthesis inhibitors (cycloheximide), and compounds that interfere with Ca transport ([D-600, α-isopropy-α-[N-methyl-N-homoveratryl]-γ-aminopropyl]-3,4,5-trimethoxy phenylacetonitrile) (Cooksey & Cooksey 1980, Cooksey et al. 1980). The formation of sulfated polysaccharides responsible as gel for the attachment of the spores of green macroalgae can be blocked by the reduction of calcium and borate supply (Haug 1976). All these experiments, however, have been carried out only under laboratory conditions and without regard to antifouling aspects.

**Competitive inhibition of receptors by offering specific lectin-like substances**

It has been shown for many macrofouling organisms that special substances (e.g. insoluble protein-conjugates) can contact with corresponding receptors of the larvae and induce an attachment and a metamorphosis. By offering specific lectin-like substances, which have a stronger affinity to the receptors than the insoluble protein-conjugates, the attachment is affected (Morse 1984). It is also possible to inhibit adhesion processes by offering simple sugars to bacterial lectins (reversed process) (Corfield & Schauer 1982, Reuter et al. 1982, Sönning 1993). These experiments have been performed only under laboratory conditions.

**Negative chemotaxis**

Numerous experimental results prove that special organic nontoxic substances (e.g. acrylamide, benzoic, tannic and sialic acids) have a negative effect on the chemotaxis of bacteria, which results in an inhibition of bacterial attachment (Chet et al. 1975, Mitchell et al. 1975, Chet & Mitchell 1976). Chemotaxis acts as a form of gravity, holding motile bacteria close to biofouling surfaces (Mitchell & Kirchman 1984). The strong negative charged molecules of sialic acid are able to hold bacteria at distance, so that fixed association with the surface is blocked up (Corfield & Schauer 1982, Reuter et al. 1982, Sönning 1993). Successful field experiments have not yet been carried out.

**Biogenic agents**

Many marine organisms produce biogenic agents with antibacterial, antialgal, antifungal, antiprotozoan and antimacrofouling properties to defend themselves against robbers and settlement in the marine environment. Therefore, the production and isolation of biogenic substances from marine organisms seem to be the most promising and effective methods for the prevention of biofouling.

**BIOCENIC AGENTS AND THEIR EFFECTS ON FOULING ORGANISMS**

**Definition of biogenic agents**

Numerous living organisms, including microorganisms, fungi, plants and animals, are able to synthesize biogenic agents. These biogenic agents are synthesized in the secondary metabolism of the producer and are not directly essential for its life. They serve animals as protection from enemies (e.g. robbers), plants as protection against feeding and microorganisms in the suppression of growth and reproduction of other microbes. Teuscher & Lindequist (1988) defined biogenic agents as follows: biogenic agents are chemical compounds which are synthesized by living organisms and which, if they exceed certain concentrations, cause temporary or permanent damage or even the death of other organisms by chemical or physicochemical effects. The concentration which causes damage and the extent of this damage are determined by the type of substance, the place and type of application, the duration of the effect, the individual sensitivity of the corresponding organism and other factors.

Marine organisms (especially micro- and macroalgae and marine invertebrates) were investigated in the last decade with growing intensity regarding chemistry and pharmacology of their active agents. The sub-
stances isolated in this connection belong, above all, to the groups of fatty acids, terpenes, terpenoids, lipoproteins, glycolipids, phenols, lactons, alkaloids and peptides. Depending on the screening methods used, the effects of the isolated potential drugs were mainly antibacterial, antiviral and antifungal. According to Ireland et al. (1993), ca 35% of these biogenic compounds are produced by micro- and macroalgae and ca 65% by marine invertebrates. The ability of marine organisms to produce biogenic substances with antibacterial, antialgal, antiprotozoan and antimacrofouling properties could be used in the prevention of biofouling.

**Biogenic agents isolated from micro- and macroalgae**

An immense number of substances with antibacterial, antiviral, antifungal and pharmacological properties have been isolated from micro- and macroalgae, analyzed, and tested for medical purposes in the last few years. Tables 1 to 5 give an overview of the existence of special biogenic compounds in micro- and macroalgae and their effect on the growth of several bacteria, algae, fungi, protozoa and macrofoulers. Several biogenic compounds, such as bromophenols, malyngolides, aponin, cyanobacteria, hapatindoles, fischellerin, galactosyl(diacylglycerols, tjipanazoles and scytophycins, isolated from special species of Cyanophyceae that have either antibacterial, antialgal, antifungal or antiprotozoan effects, are given in Table 1. Macrofoulers were not tested. Tables 2 & 3 show bioactive substances (e.g. fatty acids, glycolipids/lipoproteins, terpenes/carbohydrates, goniodomin, chlorophyll c and α-linolenic acid) isolated from special species of Chrysophyceae, Dinophyceae and Chlorophyceae that have inhibitory effects on the growth of bacteria, algae, fungi and protozoa. Macroalgae like Phaeophyceae, Chlorophyceae, Conjugata phyceae and Charophyceae also produce biogenic substances with antibacterial, antialgal, antifungal, antiprotozoan and antimacrofouling effects (Tables 4 & 5). The special biogenic substances listed in the tables were isolated from micro- and macroalgae and tested predominantly with regard to antibacterial, antialgal and antifungal activities and not with regard to antifouling aspects. Effects on the growth of macrofoulers (e.g. polychaetes, mussels) were investigated only with some species of macroalgae (Laminaria digitata, Costaria costatum, Undaria pinnatifida; Tables 4 & 5). In contrast to micro- and macroalgae, higher plants are well documented as antifouling agents (Sawant et al. 1992, Sawant & Wagh 1994).

The cultivation of micro- and macroalgae as well as the extraction of biogenic agents is expensive. However, algae can be used very well as 'extraction organisms' because they can be cultivated in a short time in mass culture independent of season and can be manipulated to a large extent in the direction of production of biogenic agents'.

<table>
<thead>
<tr>
<th>Species</th>
<th>Biogenic agent</th>
<th>Effects</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calothrix brevissima</td>
<td>Bromophenols</td>
<td>x</td>
<td>Pedersen &amp; Da Silva (1973)</td>
</tr>
<tr>
<td>Lyngbya majuscula</td>
<td>Malyngolide</td>
<td>x</td>
<td>Cardillina et al. (1979)</td>
</tr>
<tr>
<td>Gomphosphaeria aponina</td>
<td>Aponin</td>
<td>x</td>
<td>Eng-Wilmot et al. (1979)</td>
</tr>
<tr>
<td>Anabaena flos-aquae</td>
<td></td>
<td></td>
<td>Snell (1980)</td>
</tr>
<tr>
<td>Scytonema holmanna</td>
<td>Cyanobacterin</td>
<td>x</td>
<td>Mason et al. (1982)</td>
</tr>
<tr>
<td>Fischereilla muscicola</td>
<td>Methanolic extracts</td>
<td>x</td>
<td>Moore et al. (1987)</td>
</tr>
<tr>
<td>Scytonema pseudoholmanni</td>
<td>Scytophycins</td>
<td></td>
<td>Carnell et al. (1988)</td>
</tr>
<tr>
<td>Hapalosiphon fonsinatis</td>
<td>Hapalindoles</td>
<td>x</td>
<td>Baghi et al. (1990)</td>
</tr>
<tr>
<td>Synechocystis leopoliensis</td>
<td>Methanolic extracts</td>
<td>x</td>
<td>Gross et al. (1991)</td>
</tr>
<tr>
<td>Oscillatoria sp.</td>
<td>Either extracts</td>
<td>x</td>
<td>De Maitre et al. (1991)</td>
</tr>
<tr>
<td>Fischereilla musicola</td>
<td>Fischellerin</td>
<td>x</td>
<td>Bloor &amp; England (1991)</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>Methanolic extracts</td>
<td>x</td>
<td>Gromov et al. (1991)</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>Aqueous extracts</td>
<td>x</td>
<td>Murakami et al. (1991)</td>
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<tr>
<td>Nostoc linckia</td>
<td>Cyanobacterin LU-1</td>
<td>x</td>
<td>Bonjouklian et al. (1991)</td>
</tr>
<tr>
<td>Phormidium tenele</td>
<td>Galactosyl(diacylglycerols</td>
<td>x</td>
<td>Patterson &amp; Carmeli (1992)</td>
</tr>
<tr>
<td>Tolypothrix tjipanasensis</td>
<td>Tjipanazoles</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Scytonema ocellatum</td>
<td>Tolytoxin (scytophycin)</td>
<td>x</td>
<td></td>
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Table 2. Existence and effects of biogenic agents isolated from Chrysophyceae and Dinophyceae (microalgae)

<table>
<thead>
<tr>
<th>Species</th>
<th>Biogenic agent</th>
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<tr>
<td></td>
<td></td>
<td>Antibacterial</td>
<td>Antialgal</td>
</tr>
<tr>
<td>Chrysophyceae</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ochromonas danica</td>
<td>Fatty acids?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prymnesium parvum</td>
<td>Glycolipid/lipoprotein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinophyceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protogonyaulax tamarensis</td>
<td>Terpenes/carbohydrates</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Protocentrum micans</td>
<td>Terpenes/carbohydrates</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Goniodoma sp.</td>
<td>Goniodomin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peridinium bipes</td>
<td>Fatty acids, chlorophyll c</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Prorocentrum lima</td>
<td>Polyether compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinophysis torti</td>
<td>Polyether compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gambierdiscus toxicus</td>
<td>Polyether compounds</td>
<td></td>
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</tbody>
</table>

Biogenic agents isolated from marine invertebrates

In recent years several comprehensive accounts of antifungal, antibacterial, antialgal, antiviral and pharmacological activities of biogenic substances isolated from several species of marine invertebrates have been published (Targett et al. 1983, Bakus & Kawaguchi 1984, Wahl 1987, Sears et al. 1990, Ireland et al. 1993). From 1977 to 1987, 1595 bioactive compounds were isolated from marine invertebrates worldwide, predominantly sponges, coelenterates, bryozoans and ascidians (Ireland et al. 1993). Tests for medical purposes were performed, for instance, with bryostatines from bryozoans with antitumoral properties, with didemnines from ascidians with antiviral and antitumoral activities and palytoxin from corals with neurotoxic effects (Ireland et al. 1993). Furthermore, a variety of sessile marine invertebrates contain secondary metabolites affecting the settlement of fouling organisms. It is known that representatives of Porifera, Cnidaria and Tunicata are rarely overgrown by epiphytic organisms. They frequently produce high concentrations of biogenic agents with potent antifoulant activities. Especially octocorals and sponges are a rich source of compounds that act as antifoulants (Willemsen & Ferrari 1993).

Targett et al. (1983) determined that Leptogorgia virulgata and L. setacea contain high concentrations of homarine, which inhibited growth of the biofouling diatom Navicula salinicola. Extracts of L. virulgata and Neosimnia uniplicata, a snail of L. virulgata, strongly inhibited the settlement of the barnacle Balanus amphitrite. Bioassay-directed purification of L. virulgata extracts led to the identification of 2 diterpenoid hydrocarbons, pukalide and epoxypukalide, as antifouling agents (Gerhart et al. 1988). Many investigations with new species of octocorals and sponges result in the discovery of new compounds, e.g. herbacin, a new furanosequiterpene from the marine sponge Dysidea herbacea (Sarma et al. 1986).

A series of chemical compounds, like lactons, fatty acids, bromopyrroles, homarine, herbacin, pukalides,
peptides, steroids and saponins, isolated from several species of Porifera, Cnidaria, Tunicata and Mollusca with antibacterial, antialgal activities and activities preventing the settlement by macrofouling organisms are listed in Table 6. In some cases, the structure of the active agent has not yet been clarified (Standing et al. 1984, Ware 1984, Rittschof et al. 1985, Sears et al. 1990, Mary et al. 1991) (Table 6). Even the results of tests of 51 sponge extracts under field conditions for their ability to prevent biofouling showed the potential antifouling activity of most of the tested crude extracts (Willemse & Ferrari 1983) containing compounds of unknown structure. It can be seen from Table 6 that antibacterial and antialgal effects (effects against microfouling) are predominantly observed for homarine, fatty acids, peptides and steroids from Polysyncratan lacazei (ascidian) and Leptogorgia virgulata (coral) (Targett et al. 1983, Wahl 1987).

Potent antimacrofouling activity against the blue mussel Mytilus edulis and the barnacle Balanus amphitrite and other macrofouling organisms was found in extracts of several sponges (Lissodendoryx

Table 4. Existence and effects of biogenic agents isolated from Phaeophyceae (macroalgae). S.: Spirorbis, M.: Mytilus

<table>
<thead>
<tr>
<th>Species</th>
<th>Biogenic agents</th>
<th>Effects</th>
<th>Source</th>
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<td></td>
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<td>Anti-</td>
<td>Anti-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bacterial</td>
<td>algal</td>
</tr>
<tr>
<td>Laminaria digitata</td>
<td>?</td>
<td>x</td>
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<tr>
<td>Fucus vesiculosus</td>
<td>Phlorotannins</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Cystoseira baleareica</td>
<td>Lipid extract</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Zanardinia prototypus</td>
<td>Lipid extract</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Pelvetia canaliculata</td>
<td>Phlorotannins</td>
<td>x</td>
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<tr>
<td>Laminaria saccharina</td>
<td>Unsaturated fatty acids</td>
<td>x</td>
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<td>Desmarestia liqulata</td>
<td>Unsaturated fatty acids</td>
<td>x</td>
<td></td>
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<tr>
<td>Sargassum hornieri</td>
<td>Mucilage extract</td>
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<td></td>
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<tr>
<td>Coelenteria costatum</td>
<td>Glycerols</td>
<td></td>
<td></td>
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<tr>
<td>Fucus vesiculosus</td>
<td>Methanolic extract</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Fucus endentatus</td>
<td>Methanolic extract</td>
<td>x</td>
<td></td>
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<td>Fucus spiralis</td>
<td>Methanolic and chloroform extract</td>
<td>x</td>
<td></td>
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<tr>
<td>Laminaria agardhii</td>
<td>Methanolic and chloroform extract</td>
<td>x</td>
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<tr>
<td>Sargassum wightii</td>
<td>Chloroform extract</td>
<td>x</td>
<td></td>
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<tr>
<td>Padina tetraromatica</td>
<td>Chloroform extract</td>
<td>x</td>
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</table>

Table 5. Existence and effects of biogenic agents isolated from Rhodophyceae, Chlorophyceae, Conjugatophyceae and Charophyceae (macroalgae). M.: Mytilus

<table>
<thead>
<tr>
<th>Species</th>
<th>Biogenic agent</th>
<th>Effects</th>
<th>Source</th>
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<td></td>
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<td>Anti-</td>
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<td>bacterial</td>
<td>algal</td>
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<td>Laurencienyne</td>
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<tr>
<td>Centroceras clavulatum</td>
<td>Lipid extract</td>
<td>x</td>
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<td>Sphaerococcus coronopifolius</td>
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<td>x</td>
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<td>Chloroform extract</td>
<td>x</td>
<td></td>
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<tr>
<td>Acanthophora delilei</td>
<td>Chloroform extract</td>
<td>x</td>
<td></td>
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<tr>
<td>Codium coralloides</td>
<td>Lipid extract</td>
<td>x</td>
<td>x</td>
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<td>Caulerpa asheaddi</td>
<td>Terpenoids</td>
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<td>Undaria pinnatifida</td>
<td>Glycerols</td>
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<td>M. edulis</td>
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<td>Enteromorpha linza</td>
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<td>Spirogyra sp.</td>
<td>Tannin ?</td>
<td>x</td>
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<tr>
<td>Chara globularis</td>
<td>Dithiolan, thrihan</td>
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Table 6. Existence and effects of antifouling agents isolated from special species of marine invertebrates. Species: Agaricia lamarcki, Balanus amphitrite, Bugula neritina, Hippopora americana, Hydroides norvegica, Laomedia bistriata, Mytilus edulis, Serpula vermicularis

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<th>Species</th>
<th>Biogenic agent</th>
<th>Anti-</th>
<th>Anti-</th>
<th>Antimacroc-</th>
<th>Source</th>
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<td><strong>Porifera</strong></td>
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<td>Xestospongia halichondriodes</td>
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<tr>
<td>Placortis halichondriodes</td>
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<tr>
<td>Lissodendoryx isodictyalis</td>
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<tr>
<td>Agelas conifera</td>
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<tr>
<td>Dysidea herbacea</td>
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<tr>
<td><strong>Phyllospongia papyracea</strong></td>
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<td>Cnidaria (Anthozoa)</td>
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<td>Leptogorgia setacea</td>
<td>Lactons, phenols, cyclic peroxides</td>
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<tr>
<td>L. virgulata</td>
<td>Lactons, phenols, cyclic peroxides</td>
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<td>L. virgulata</td>
<td>Terpenoids?</td>
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<td>Herbicin (furansosquinoterpenes)</td>
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<td>Renilla reniformis</td>
<td>Terpenoids?</td>
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<tr>
<td>L. virgulata</td>
<td>Terpenoids?</td>
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<td>Suberogorgia suberosa</td>
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<td>Spongodes sp.</td>
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<td>Solenocaulon tortuosum</td>
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<td>Echinogorgia complexa</td>
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<td>Juncella juncea</td>
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<td><strong>Mollusca (Gastropoda)</strong></td>
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<td>Neosimnia uniplicata</td>
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<tr>
<td><strong>Chordata</strong> (Asciacea)</td>
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<tr>
<td>Polysyncrator lacazei</td>
<td>Fatty acids, peptides, steroids</td>
<td></td>
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</table>

isodictyalis, Phyllospongia papyracea, Agelas conifera, Dysidea herbacea) and corals (e.g. Leptogorgia virgulata, L. setacea, Renilla reniformis, Suberogorgia suberosa, S. tortuosum and others) (Table 6).

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stances isolated from aquatic macrophytes (Charales). Oikos 39:187–190

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Furness, R. W.
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Mechanisms of cell death: resistance and control

CULTIVATION

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Birkeland, C.
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Brattegard, T.
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Brownman, H. I.
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Dolan, M. @
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Structure and function of fish and benthic communities

Feller, R. J.
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Trophic dynamics and energetics of crustaceans; feeding and digestion of fishes; benthic ecology of meiofauna; ecological applications of immunology and ontogeny

Fenchel, T. @
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Field, J. G.
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Dynamics of nearshore ecosystems

Floc’h, J. Y.
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