REVIEW ARTICLE

Natural product antifoulants

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Natural Product Antifoulants (NPAs) have been proposed as one of the best replacement options for the most successful antifouling agent, tri-*n*-butyl tin (TBT), which, due to its ecological incompatibility, is currently facing total global ban imposed by International Maritime Organization (IMO). Realizing the importance, commercial and industrial, of immediately finding a suitable replacement for TBT, the research on NPAs has gathered considerable momentum during the last two decades, as evidenced from the sudden spurt in the number of publications and the number of NPAs being reported. Although commendable effort has been expended, more challenges remain ahead before realizing their applications at an industrial scale.

Keywords: Antifouling, biofouling, natural products, secondary metabolites, TBT.

MARINE biofouling, the attachment and growth of sessile organisms on artificial structures submerged in the sea, incurs substantial financial implications to the marine engineering constructions comprising ships, offshore platforms, jetties and harbours[1–4]. The problem is so severe that worldwide the expenditure incurred on antifouling measures alone is approximately US$ 6.5 billion a year[5]. Application of antifouling coatings has been a widely employed strategy for controlling fouling on underwater marine structures. Among the various coatings, Self-Polishing Copolymer antifouling paints (SPCs) with tributyl tin (TBT) as biocide was the most preferred[6]. Thus, 70% of the world's shipping fleet was coated with self-polishing TBT paints[7], accounting for two-thirds of the total antifouling market[8] in the 1990s. Unfortunately, this most popular antifouling coating, having a life time up to five years, also turned out to be the most toxic[9]. Excessive introduction of this biocide into the environment caused shell thickening in oyster population and imposex in gastropods[10–13]. Further, their build-up in the marine food chain through bioaccumulation and biomagnification attracted utmost concern. The subsequent total global ban imposed on TBT-based coatings by the International Maritime Organization (IMO) led the antifouling paint industry into a very precarious situation[14]. Although copper and organic booster biocides blended with copolymers have widely been used as immediate alternatives for TBT, considering their ecotoxicity, durability and cost factors, most of these biocides might be considered as interim solutions rather than real alternatives for TBT[15,16].

TBT-replacement antifouling coatings, therefore, have to be environmentally acceptable and at the same time, should maintain a long life[6]. Natural Product Antifoulants (NPAs) have been proposed as one of the best possible alternatives in this context[6,15,17,18]. The NPAs are advantageous over conventional toxic biocides in that they are less toxic, effective at low concentrations, biodegradable, have broad spectrum antifouling activity and their effects are reversible[19]. Moreover, they have evolved within the system through millions of years of evolution. NPAs, especially those having anaesthetic, repellent or settlement inhibition properties, without being biocidal, are the most desired[15]. In general, the search for NPAs is greatly encouraged by the fact that the effect of these compounds is based more on repellent mode of action than on strong toxicity.

The majority of NPAs identified so far are terpenoids, steroids, carotenoids, phenolics, furanones, alkaloids, peptides and lactones. They have been isolated from a wide range of organisms of which the major groups are represented by sponges[20–22] and soft corals[23–25], as they are well known for maintaining foul-free surfaces[26]. Other groups include seaweeds, seagrasses[27–29], tunicates[30,31], bryozoans[32,33], mangroves and microorganisms[34–36]. In recent studies, crustaceans such as lobster and shore crabs, echinoderms such as sea stars and sea urchins, as well as egg cases of molluscs and dogfishes were investigated to elucidate their antifouling strategy[37–39]. Accordingly, over 145 NPAs have thus far been isolated and identified from marine sources (Tables 1–5).

In general, a sudden spurt in the number of publications on NPAs in 1990s and 2000s was evidently a consequence of the anticipated ban on TBT by IMO beginning January 2008 (Figure 1).

Present scenario

The search for bioactive compounds from marine organisms was initiated[27] in the 1960s. The possibility of collecting organisms directly from the ocean with the use of SCUBA opened the door to a largely untapped resource with a range of unique structures and novel compounds. The common methodology employed in the isolation of

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Table 1. Antifouling activity detected in sponges

Sponge species	A/F compound	Activity	Laboratory	Year
Aaptos sp.	Aaptamine	Z.m.	Univ M, USA	2006
Acanthella acuta	Crude extract	*B.n.*		1993
Acanthella cavernosa	Bact. extract	*B.a.*	FBP, Japan	1995
	Kalihipyran B, 15-formamido Kalihinene, kalihinol A, 10β-formamidokalihinol A, 10-isocyano-4-cadinene, isocyanotheonellin	*B.a.*	FBP, Japan	1996
	Steroid peroxidase	*B.a.*	FBP, Japan	1998
	10-formamide-4-cadinene	*B.a.*	FBP, Japan	2003
	Isocyanide	*B.a.*	ARL, Japan	2004
	Kalihinol A	*H.e.*	CML, China	2006
Agelas conifera	*Bromopyrroles*	*B. a.*		1991
Agelas mauritiana	Mauritiamine	*B.a.*	FBP, Japan	1996
	Epi-agelasine C	*U.c.*	MB I, Japan	1997
Agelas sp.	Agelasine D	*B.i.*	Uppsala Univ Sweden	2008
Aplysilla glacialis	1-Methyl adenine	Ab		1992
Aplysina fistularis	Aerothionin, Homoaerothionin	*H.r.*		1985
Axinella sp.	Isonitriles, Isothiocyanates	*P.pa.*; *S.t.*; *H.r.*		1985
Axinyssa sp.	Axinyssimide A, B, C; 2-chloro-10,11-dihydroxy-3-methylene-7,11-dimethyl-6-dodecenyl-dicholromethylenamine	*B.a.*	FBP, Japan	1998
Azorica pfeifferae	Crude extract	*B.a.*	RRL, Orissa	1999
Cacospongiascalaris	Dihydrofurospongin-II	*B.a.*	NC Univ, UK	2005
Callyspongia sp. 1	Crude extract	Bact.; Dtm.		2005
Callyspongia sp. 2	Crude extract	Bact.; Dtm.		2005
Callyspongia plicifera	Crude extract	*B.a.*	CML, China	2006
Callyspongia pulvinata	Crude extract	Bact.; Dtm.		2005
Callyspongia truncata	Calytetrayne, Callyspongin B, Callytriol C	*B.a.*	FBP, Japan	1997
Crambe crambe	Crambescins	*B.n.*		1997
Craniella sp.	Crude extract	*B.n.*		1993
	Crude extract	Ab	NIO, Goa	1994
	Crude extract	*N.su.*; *N.c.*		1994
Crella incrustans	*lyso*-PAF	B.n.		1996
Dendrilla herbacea	Herbacin	B.n.	Vizag	1991
Dendrilla nigrae	Crude extract	Af	Biotechnology Dept, Kanyakumari	2004
Dysidea amblia	Ambliol-A, Pallescensin-A	*S.t.*; *H.r.*		1985
Dysidea avara	Avarol	*B.a.*	Univ Athens, Greece	2007
Dysidea sp.	Crude extract	*B.a.*	NC Univ, UK	2005
Erylus formosus	Formoside	F.i.; Algae		2000
Geodia barrette	Barettin	*B.i.*	Uppsala Univ, Sweden	2006
Halichondria sp.	Crude extract	Bact.; Dtm.		2005
Haliclona sp.	Cyclic *bis*-(3-alkylpiperidine) Haliclonamides	*H.c.* *M.e.*	MBI, Japan	2002
Haliclona sp.	Crude extract	*P.vi.*	NIO, Goa	1998
Haliclona cymaeformis var. 1	Haliclonamides	Bact.; Dtm.		2005
H. cymaeformis var. 2	Crude extract	Bact.; Dtm.		2005
Haliclona? Cinerea	Undescribed mixture	*P.pa.*; *S.t.*; *H.r.*		1985
Haliclona exigua	*bis*-1-oxaquinolizidine alkaloids	*B.a.*, F.bact	NIO, Kochi	2009

(*Contd*)

Table 1. (*Contd*)

Sponge species	A/F compound	Activity	Laboratory	Year
Ianthella basta	Bastadins	*B.i.*	Heinrich-Heine Univ, Germany	2006
Ircinia fasciculata	Crude extract	Ab		1994
Ircinia oros	Mixture of Ircinin I & II	Macroalgae	Univ Athens, Greece	2002
Ircinia ramosa	Crude extract	F.bact.	NIO, Goa	2000
Ircinia spinosula	Hydroquinone A	Macroalgae	Univ Athens	2002
	Hydroquinone-C acetate	*B.a.*	NC Univ, UK	2005
Ircinia variabilis	Crude extract	Macroalgae	Univ Athens	2002
Leiosella idia	Idiadione	*S.t.*; *H.r.*		1985
Mycale adherens	Heteronemin, 12-epi-deoxoscalarin, deacetyl-12,18-diepiscalaradiol, Scalarafuran	*H.r.*		2005
Pachychalina lunisimilis	Undescribed mixture	*S.t.*; *H.r.*		1985
Petrosia sp.	Crude extract	F.bact.	NIO, Goa	2002
Phyllospongia papyracea	Fatty acid	*M.e.*	FBP, Japan	1996
Placortis halichondroides	*Lactones*, *phenols*, *cyclic peroxides*	*A.l.*		1988
Protophlitaspongia aga	Pyrimidine derivative, Zooanemonin, α-nicotinamide ribose	*B.a.*	MBI, Japan	2001
Psammaplysilla purpurea	Aplysillamide A, B	Antimicrobial	Japan	1995
	Bromotyrosine alkaloids	F.bact.	NIO, Goa	2004
Pseudoceratina purpurea	Ceratinamine, ceratinamide A, B; Psammaplysin A, Morokaiamine, Moloka'iamine 1, 3,5-dibromo-4-methoxy-phenethylamine 2, Pseudoceratidine	*B.a.*	FBP, Japan	1996
	Bromotyrosine derivative	F.bact. (*R.s.*)	Japan	2001
	Zammamistatin	Ab	Japan	2001
Reneira sarai	Poly APS	*B.a.*	CNR, Italy	2003
Spongia officinalis	Crude extract	Ab		1994
Stylotella aurantium	Styloguanidine	*B.a.*		1995
Toxadocia zumi	Sterol sulphates	*S.t.*		1985

Z.m., Zebra mussel; *B.n.*, *Bugula neritina*; *B.a.*, *Balanus amphitrite*; *H.e.*, *Hydroides elegans*; *U.c.*, *Ulva conglobata*; *B.i.*, *Balanus improvisus*; Ab, Antibacterial; *H.r.*, *Haliotis rufescens*; *P.pa.*, *Phidolophora pacifica*; *S.t.*, *Salmacina tribranchiata*; Bact., Bacteria; Dtm., Diatom; *N.su.*, *Navicula subinflata*; *N.c.*, *Navicula crucicula*; Af, Antifouling; F.i, Fouling invertebrates; *H.c.*, *Herdmania curvata*; *M.e.*, *Mytilus edulis*; *P.vi.*, *Perna viridis*; *A.l.*, *Agaricia lamarcki*; F.bact., Fouling bacteria; *R.s.*, *Rhodospirillum salexigens*; MBI, Marine Biotechnology Institute; NIO, National Institute of Oceanography; Univ, University; NC Univ, Newcastle University; FBP, Fusetani Biofouling Project; CNR, Consiglio Nazionale delle Ricerche; ARL, Abiko Research Lab; Univ M, University of Massachussets; Vizag, Vishakapattnam.

antifouling compounds is solvent extraction followed by bioassay-guided fractionation and purification[40,41]. Thereafter, spectroscopic analysis is carried out for structure elucidation of the isolated compound (Figure 2). The commonly used antifouling assay organisms include fouling bacteria and microalgae, and barnacle cyprids as representatives of the microfouling and macrofouling communities respectively. The antifouling property of the NPA is assessed using 'growth inhibition assay' for fouling bacteria and microalgae and 'settlement inhibition assay' for barnacle cyprids[23,41–45]. Subsequently, the effective concentration causing 50% inhibition of the test individuals within a specified time period (EC_{50}) and the lethal concentration causing 50% death of the test individuals within a specified time period (LC_{50}) of the NPA are determined. An EC_{50} of 25 μg/ml or less is the standard set by the US Navy as an efficacy level for NPAs[46]. From the above observations, the calculated therapeutic ratio (LC_{50}/EC_{50}) of greater than 1 is an essential prerequisite for potential use of the NPA in environmentally compatible antifouling coatings[47].

Presently, intensive search for NPAs as a natural non-toxic means of fouling control is ongoing in various laboratories worldwide.

Sponges

In spite of having the least differentiated body-plan of all Metazoa, sponges have long been the centre of attention for natural product chemists because they produce a wide array of secondary metabolites, many of unusual chemistry, and often in high concentrations with potent bioactivities[48–50]. This is further substantiated by the fact that more

Table 2. Antifouling activities detected in soft corals

Softcoral species	A/F compound	Activity	Laboratory	Year
Acanthogorgia turgida	Crude extract	*B.a.*; *P.vi*	RRL, Orissa	1998
Alcyonium paesslerii	Crude extract	Bact.	Univ Mississippi, USA	1995
Cladiella pachyclados	Crude extract	*P.vi*	NIO, Goa	1998
Cladiella sp.	(–)-6α-hydroxy polyanthellin	*B.a.*	NIO, RC, Kochi	Unpl
Dendronephthya sp.	12α-acetoxy-13,17-seco-cholesta-1,4-dien-3-ones	*B.a.*	JRDC, Japan	1999
	partially purified extract	*M.e.*		1993
Dendronephthya sp.	Crude extract	*B.a.*; *P.vi.*	RRL, Orissa	1998
Echinogorgia complexa	Crude extract	*B.a.*; *P.vi*	RRL, Orissa	1998
	Crude extract	*B.a.*	SHMRC, Tuticorin	1991
Echinomuraceae splendens	Caffeine	Dtm.	NIO, Goa	2002
Eunicea sp.	Isothiozolone	Ent.	California, USA	1981
Euplexaura nuttingi	Crude extract	*B.a.*	SHMRC, Tuticorin	1991
Gersemia antarctica	Crude extract	Bact.	Univ Mississippi, USA	1995
Gorgonella sanguinolenta	Crude extract	*B.a.*; *P.vi*	RRL, Orissa	1998
Juncella juncea	Juncins	*B.a.*	SCSIO, China	2006
	Partially purified extract	*B.a.*	SHMRC, Tuticorin	1991
	Juncellins	Bact.	SHMRC, Tuticorin	1993
Leptogorgia virgulata	Homarine	*N.sa.*	Sk.I.O., Georgia	1983
	Crude extract	*B.a.*	BML, California	1984
	Partially purified extract	*B.n.*	Duke Univ. Mar. Lab.,	
	Pukalide; epoxypukalide	*B.a.*	N. Carolina	1988
Lobophytum pauciflorum	Diterpenes	*C.f.*	J.C. Univ N. Q., Australia	1987
Lobophytum sp.	Diterpenes	B. larvae	RRIMP–NSW, UK	1979
Mauricea fruticosa	Muricins	*P.t.*	Sc.I.O., USA	1985
Phyllogorgia dilatata	11β,12 β-Epoxypukalide	*P.pe.*	U.F.F., Brazil	2006
Pseudopterogorgia Americana	Partially purified extract	*N.* sp.	Sk.I.O., Georgia	1988
	Furanogermacrene	Dtm.		1989
Pseudopterogorgia acerosa	Partially purified extract	*N.* sp.	Sk.I.O., Georgia	1988
Renilla reniformis	Partially purified extract	*B.n.*	Duke Univ. Mar. Lab., N. Carolina	1988
Renilla reniformis	Renillafoulin A	*B.a.*	Univ of Illinois, USA	1986
Sclerophytum sp.	Crude extract	*B.a.*	SHMRC, Tuticorin	1991
Sinularia compressa	Crude extract	*B.p.*; *P.ve*	NIO, Goa	2002
Sinularia flexibilis	Sinulariolide; 11-episinulariolide	*C.c.*	J.C. Univ N. Q., Australia	1997
Sinularia kavarattiensis	Furoic acid	*B.a*	NIO, RC, Kochi	Unpl
Sinularia numerosa	Crude extract	*P.vi.*	NIO, Goa	1998
Sinularia sp.	(–)-β-bisabolene	*M.e.*	MBI, Japan	1993
	13α-acetoxypukalide and (9E)-4-(6,10-dimethylocta-9-11-dienyl)-furan-2-carboxylic acid	*B.a.*; *M.e.*		1994
Solenocaulon tortuosum	Crude extract	*B.a.*	SHMRC, Tuticorin	1991
Spongodes sp.	Crude extract	*B.a.*	SHMRC, Tuticorin	1991
Subergorgia reticulata	Calamenenes	*B.a.*	NIO, RC, Kochi	Unpl
Subergorgia suberosa	Crude extract	*B.a.*	SHMRC, Tuticorin	1991
	Crude extract	*P.vi.*	NIO, Goa	1998
Xenia elongata	Crude extract	Bact.		2002
Xenia macrospiculata	Desoxyhavannahine	Bact.		2005

N.sa., *Navicula salinicola*; *C.f.*, *Ceramium flaccidum*; B. larvae, Barnacle larvae; *P.t.*, *Phaeodactylum tricornutum*; *P.pe.*, *Perna perna*; *N.* sp., *Navicula*; *B.p.*, *Bacillus pumilus*; *P.ve.*, *Pseudomonas vesicularis*; *C.c.*, *Ceramium codii*; RRL, Regional Research Lab; JRDC, Research Development Corporation of Japan; SHMRC, Sacred Heart Marine Research Centre; SCSIO, South China Sea Institute of Oceanology; Sk.I.O., Skidaway Institute of Oceanography; BML, Biodega Marine Laboratory; J.C. Univ N.Q., James Cook University of North Queensland; RRIMP, Roche Research Institute of Marine Pharmacology; U.F.F., Universidade Federal Fluminense.

than 50% of the NPAs isolated to date are from sponges (Figure 3). Yet sponges remain as one of the most underexplored group of organisms (hardly fewer than 100 species out of more than 10,000 described species have been investigated) as far as NPAs research is concerned (Table 1).

Sponges are soft-bodied organisms, and as such cannot physically defend themselves from predators and com-

Table 3. Antifouling activity detected in seaweeds

Seaweed species	A/F compound	Activity	Laboratory	Year
Ascophyllum nodosum	Phlorotannins	*L.c.*; *V.m.*	NC Univ, UK	1975
	Fraction	Ab, Af, Dtm., Mcalga, Musl		2004
Bifurcaria bifurcata	Linear diterpenes	B.cyp; F.bact.	France	2004
Bryothamnion seaforthii	Crude extract	*P.pe.*	CUMS, Brazil	2007
Chrondus crispus	Fraction	Ab, Af, Dtm., Mcalga, Musl	NC Univ, UK	2004
Costaria costata	Galactosyl and sulpho-quinovosyl-diacylglycerols	*M.e.*		1990
Delisea pulchra	Furanones	*B.a.*; Swd	Australia	1995
Dictyota menstrualis	Dictyol E, Pachydictyol A, Dictyodial	*B.n.*		1995
Dictyota pfaffii	Diterpene	Af	Brazil	2007
Dilophus okamurai	Spatane diterpene	Abalone larvae		1989
	Diterpene	Antimacfl		1990
Ecklonia radiata	Phlorotannins	*U.l.*		1997
Ectocarpus siliculosus	Fraction	Ab, Af, Dtm., Mcalga, Musl	NC Univ., UK	2004
Enteromorpha intestinalis	Fraction	Ab, Af, Dtm., Mcalga, Mussel	NC Univ, UK	2004
Fucus spiralis	Phlorotannins	*V.m.*		1975
Grateloupia turuturu	Isethionic acid	*B.a.*	NC Univ, UK	2004
Ishige sinicola	Crude extract	*E.p.*; *M.e.*	PNU, Korea	2001
Jania rubens	Crude extract	*P.pe.*	CUMS, Brazil	2007
Laurencia obtusa	Elatol	Musl	Brazil	2002
	5β-Hydroxyaplysistatin; Palisol; Palisadin A	*B.n.*; *B.a.*; *U.* sp.		1998
Laurencia pinnatifida	Crude extract	*R.m.*; A. sp.	France	2001
	Fraction	Ab, Af, Dtm., Mcalga, Musl	NC Univ, UK	2004
Laurencia rigida	Elatol, deschloroelatol	*B.n.*; *B.a.*	Brazil	2002
Laurencia sp.	Elatol	*B.a.*	Australia?	1996
Padina tetrastomatica	Sulpho-quinovosyldiacylglycerols	*B.p.*, *P.ve.*	NIO, Goa	2002
	Crude extract			
Plocamium costatum	Halogenated monoterpene	*B.a.*		1999
Polysiphonia lanosa	Crude extract	*E.i.*; *U.l.*	France	2001
	Fraction	Ab, Af, Dtm., Mcalga, Musl	NC Univ, UK	2004
Ralfsia spongiocarpa	Phlorotannins	Red algae		1975
Rhodymenia paimata	Floridoside	*B.a.*		2001
Sargassum horneri	Crude extract	*E.p.*	Dept of Biotech.	2001
Sargassum muticum	Crude extract	*R.m.*; *A.c.*; *E.i.*, UI	France	2001
	Fraction	Ab, Af, Dtm., Mcalga, Musl	NC Univ, UK	2004
Sargassum natans	Phlorotannins	Bact., mar. worms		1965
Sargassum vestitum	Phlorotannins	*U.l.*		1997
Scytosiphon lomentaria	Crude extract	*M.e.*	Dept of Biotech.	2001
	Phlorotannins	*V.m.*		1975
Ulva lactuca	Fraction	Ab, Af, Dtm., Mcalga, Musl	NC Univ, UK	2004
Undaria pinnatifida	Galactosyl and	*M.e.*		1990

L.c., *Laminaria cloustoni*; *V.m.*, *Vorticella marina*; Mcalga, Macroalga; Musl, Mussel; B.cyp, Barnacle cyprid; Swd, Seaweed; *U.l.*, *Ulva lactuca*; *E.p.*, *Enteromorpha prolifera*; *U.* sp., *Ulva* sp.; *R.m.*, *Rhodella maculata*; *A.c.*, *Amphora coffeaeformis*; *E.i.*, *Enteromorpha intestinalis*; CUMS, Centro University Monte Serrat; PNU, Pukyong National University.

petitors. To compensate for this, sponges produce an array of chemical metabolites to protect themselves from possible dangers[51]. Also, since sponges are voracious filter feeders, they take in along with regular food, the toxic chemicals secreted by other plants and animals, such as corals. They then modify and reuse these chemicals for their defense purposes. Thus, these sponge-produced and sponge-modified metabolites form the chemical defense mechanism of sponges[52].

Possible antifouling properties of compounds isolated from sponges was first recognized by Bakus *et al.*[53]. Since then, a lot of research has been focused in this direction. However, a major breakthrough in the isolation of NPAs from sponges was achieved in the 1990s by the

Table 4. Antifouling activities detected in Ascidians

Ascidian species	Compound	Activity	Laboratory	Year
Amaroucium stellatum	Crude extract	*B.a.*	UK	2003
Botryllus planus	Crude extract	*B.a.*	UK	2003
Clavelina lepadiformis	Crude extract	Invert.	UK	1995
Cynthia savignyi	Crude extract	*A.s.*	Morocco	1999
Distaplia nathensis	Crude extract	Af	India	2003
Eudistoma olivaceum	Eudistomins	*B.n.*	USA	1990
Eudistoma sp.	Analog of Moroka'iamine	B. larvae		1999
Halocynthia roretzi	Lysophophatidylinositols	Af; antifungal		1997
Polysyncraton lacazei	Fractions	S.U. larvae	France	1991
Pyura pallida	Crude extract	*A.s.*; Microalg	India	1989
Styela pigmentata	Crude extract	*A.s.*; Microalg	India	1989

Invert, Invertebrates; *A.s.*, *Artemia salina*; S.U. larvae, Sea Urchin larvae.

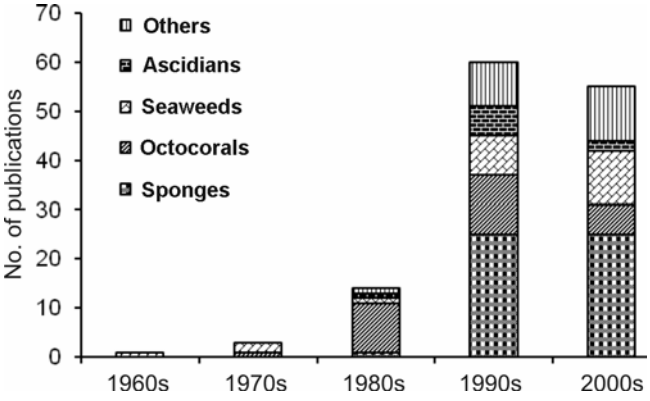

Figure 1. Published studies on antifouling research from marine organisms.

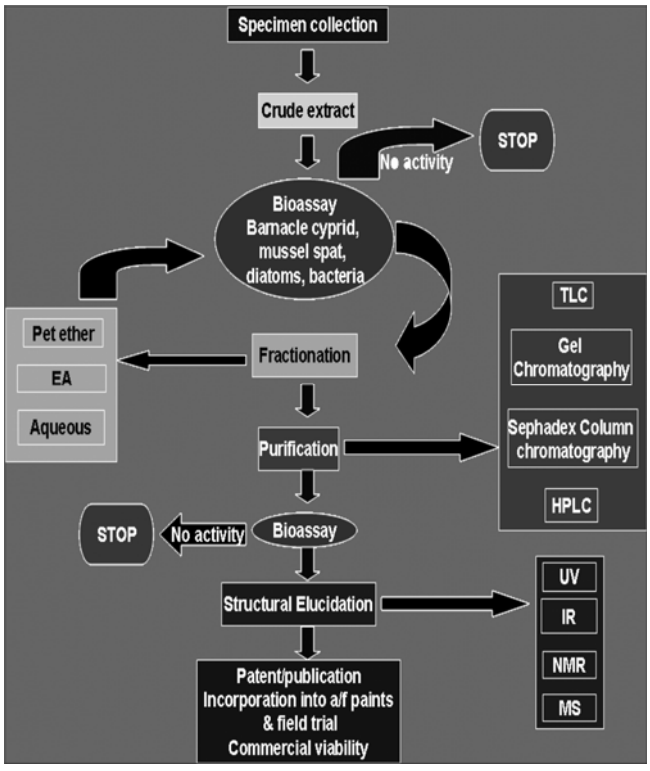

Figure 2. Activity chart.

team of researchers of the Fusetani Biofouling Project, Japan under the leadership of Nobuhiro Fusetani. They isolated terpenoids such as kalihinene and 10 β-formamidokalihinol A from the marine sponge *Acanthella cavernosa* and axinyssimide A, B and C from *Axinyssa* sp.; steroid peroxidase from *A. cavernosa*; fatty acid derivative, callytriol C, from *Callyspongia truncata*; bromotyrosine derivatives such as ceratinamide A and psammaplysin from *Pseudoceratina purpurea* and heterocyclic compounds such as pseudoceratidine from *P. purpurea* and mauritiamine from *Agelas mauritiana*. All the above-mentioned compounds were reported to inhibit the settlement of the cosmopolitan biofouler, *Balanus amphitrite*. Of these, 10 β-formamidokalihinol A was the most effective, with an EC_{50} of 0.05 μg/ml and LC_{50} > 100 μg/ml indicating its low toxicity towards barnacle cyprids[40,54–62].

Apart from the Fusetani group, search for NPAs from sponges has also been pursued at various other laboratories worldwide. These include Consiglio Nazionale delle Ricerche, Italy; Department of Biology, Slovenia; Department of Pharmacognosy and Chemistry of Natural Products, University of Athens, Greece; Coastal Marine Laboratory, China; Marine Biotechnology Institute, Japan; Duke University Marine Laboratory, North Carolina. The team of researchers at Uppsala University, Sweden has also contributed to sponge antifouling research. According to them, agelasine D from the sponges of the genus *Agelas*, and its two analogues (AV1003A and AKB695), displayed strong inhibitory effect on the settlement of cyprids of *Balanus improvisus*. Agelasine D had an EC_{50} value of 0.11 μM while the two analogues AV1033A and AKB695 had EC_{50} values of 0.23 and 0.3 μM respectively. None of these three compounds caused larval mortality[50]. Recently, antifouling *bis*-1-oxaquinolizidine alkaloids have been isolated from the sponge, *Haliclona exigua* at the National Institute of Oceanography, India[41]. More than 70 NPAs have thus been isolated from marine sponges like *A. cavernosa*, *Agelas mauritiana*, *Aplysina fistularis*, *Axinyssa* sp., *Callyspongia truncata*, *Crambe crambe*, *Crella incrustans*,

Table 5. Antifouling activities detected in miscellaneous groups

Source organism	Compound	Activity	Laboratory	Year
Bryozoans				
Amathia winsoni	Crude extract	Bact.		1993
Orthoscuticella ventricosa	Crude extract	Bact.		1993
Sinupetraliella litoralis		*H.c.*		2003
Zoobotryon pellucidum	TBG	B. larvae	MBI, Japan	1994
Seagrass				
Zostera marina	Zosteric acid	B. cyprid, Bact., algae, tbwrm	UK	2002
Mangrove species				
Ceriops tagal	Methoxy-ent-8(14)-pimarenely-15-one ent-8(14)-pimarene-15R,16-diol stigmasterol, β-sitosterol	*B.a.*	Dept of Biology, China	2008
Bacteria				
Alteromonas sp. (from *Halichondria okadai*)	Ubiquinone-8	B. larvae		1995
FS-55	Crude extract	F. bact.	HW Univ, UK	2000
NudMB50-11	Crude extract	F. bact.; *B.a.*; *U.l.*		2003
Pseudoalteromonas tunicata		*B.a.*; *C.i.*	Australia	1998
Streptomyces fungicidicus	Diketopiperazines	*B.a.*	CML, Hong Kong	2006
Vibrio spp. (epiphytic on *Dendronephthya* sp.)	Polysaccharides	Polych.; Bry.		2003
Cyanobacteria				
Calothrix brevissima	Crude extract	*N.p.*		1999
Nostoc commune	Comnostins	*S.e.*		2000
Scytonema hofmanni	Cyanobacterin	*N.p.*		1999
Fungi				
Cladosporium sp.	Sec. met	*B.a.*		2006
Ampelomyces sp.	3-Chloro-2,5-dihydroxybenzyl alcohol (CHBA)	Bact.; *B.a.*		2006
Arthrinium c.f. saccharicola	Sec. met	*B.a.*		2006
Nudibranchs				
Phyllidia krempfi	Sesquiterpene peroxide	*B.a.*	FBP, Japan	1996
Phyllidia pustulosa	Sesquiterpenes	*B.a.*	FBP, Japan	1998
Gastropods				
Trimusculus reticulatus	Labdane diterpene	*P.c.*		1996
Sea anemones				
Condylactis gigantea	Crude extract .	Alg.		1984
Nemertines				
Haplonemertines	Nemertine pyridyl alkaloids	*B.a.*		2003
Echinoderms				
Astrocyclus caecilian	Crude extract	*H.i.*	USA	2006
Astropecten articulatus	Crude extract	*H.i.*	Univ of Alabama	2006
Holothuria leucospilota	Crude extract	*N.su.*; *N.c.*	NIO, Goa	1994
Holothuria scabra	Crude extract	*P.vu.*	CMFRI, Tvm	2002
Luidia clathrata	Crude extract	*H.i.*	Birmingham	2006

tbwrm, Tubeworm; *C.i.*, *Ciona intestinalis*; Polych., Polychaete; Bry., Bryozoan; S.e., *Staphylococcus epidermis*; *P.c.*, *Phragmatopoma californica*; Alg., Algae; *H.i.*, *Hincksia irregularis*; *P.vu.*, *Patella vulgata*; HW Univ, Heriot Watt University; CML, Coastal Marine Laboratory; CMFRI, Central Marine Fisheries Research Institute.

Dysidea avara, *Dysidea herbacea*, *Erylus formosus*, *Geodia barretti*, *Haliclona exigua*, *H. koremella*, *Haliclona* sp., *Phyllospongia papyracea*, *Protophlitaspongia aga*, *P. purpurea*, *Reniera sarai* and *Stylotella aurantium* (Table 1).

Soft corals

The investigations on antifouling properties of soft corals gained momentum in the 1980s with many laboratories the world over focusing research in this direction. Scripp's

Institute of Oceanography, Duke University Marine Laboratory and University of Illinois, USA; James Cook University of North Queensland, Australia; Marine Biotechnology Institute, Japan, and South China Sea Institute of Oceanology are some of the major institutions working on the isolation of NPAs from soft corals. Homarine from *Leptogorgia virgulata* and *Leptogorgia setacea*, muricins from *Muricea fruticosa*, renillafoulins from *Renilla reniformis*, pukalide and epoxypukalide from *L. virgulata*, 11-episinulariolide and sinulariolide from *Sinularia flexibilis*, 12α-acetoxy-13,17-*seco*-cholesta-1,4-dien-3-ones from *Dendronephthya* sp., juncins from *Juncella juncea*, etc. are some of the major NPAs isolated from soft corals[23–25,63–66]. Scientists at Sacred Heart Marine Research Centre (SHMRC), Tuticorin, in collaboration with Poseidon have isolated an antifouling compound juncellin from the soft coral, *J. juncea*[67]. The NPAs isolated from soft corals are presenetd in Table 2.

Seaweeds

To date, the halogenated furanone from the red seaweed *Delisea pulchra* isolated by Stefan Kjelleberg and Peter Steinberg of the Centre for Marine Bifouling and Bioinnovation, Australia, has proved the most successful, with EC_{50} being as low as 0.02 μg/ml and their activities comparable to, or even better than, those obtained with commercial biocides[29]. Also, dictyols from *Dictyota menstrualis* and sesquiterpenes from *Laurencia rigida* inhibit the settlement of macrofoulers such as *Bugula neritina* and *Bugula amphitrite*[68,69]. The antifouling activity detected in seaweeds is listed in Table 3.

Ascidians

The NPAs isolated from ascidians are listed in Table 4, the major ones being eudistomins and lysophosphatidy linositols from *Eudistoma olivaceum* and *Halocynthia roretzi* respectively[70].

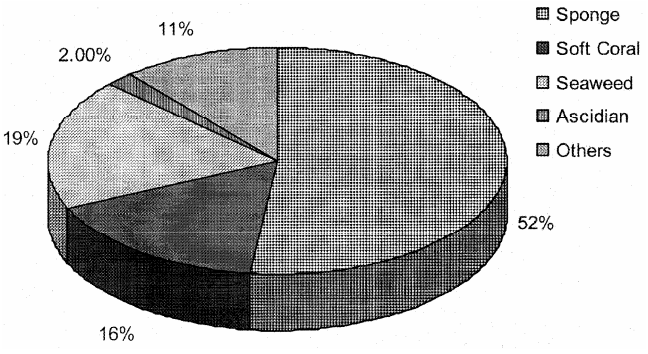

Figure 3. NPAs isolated from marine sources.

Miscellaneous

Bryozoans, nemerteans, molluscs, echinoderms, seagrasses, mangroves, microorganisms, etc. have also been explored for the presence of NPAs. The major findings are presented in Table 5.

Bryozoans: Tribromogramine (TBG) isolated from the bryozoan *Zoobotryon pellucidum* by Wataru Miki at the Marine Biotechnology Institute in Tokyo is a promising NPA. TBG is only one-tenth as toxic as TBT, but is 6–8 times as potent when it comes to the inhibition of larval settlement[33].

Nemerteans: The nemertine pyridyl alkaloids from the marine Haplonemertines have potent antifouling activity against the larvae of the barnacle *B. amphitrite*[71].

Molluscs: The marine molluscs, *Nerita albicilla* and *N. oryzarum* from Tuticorin showed broad spectral inhibitory activity against biofilm bacteria[72]. The extract of molluscan egg case also exhibited antifouling activity against fouling bacteria[39].

Echinoderms: The crude extracts of holothurians have been reported to exhibit antifouling properties. For example, the methanol extract of *Holothuria leucospilota* inhibited the fouling diatoms, *Navicula subinflata* and *N. crucicula*[37]. Crude extract of *H. scabra* also showed antifouling activity against the limpet, *Patella vulgata*[73].

Seagrasses: Zosteric acid from the seagrass, *Zostera marina* was found to inhibit micro- and macro-fouling organisms[74].

Mangroves: There is scant information on isolation of antifouling compounds from mangrove species. One new diterpene, methoxy-ent-8(14)-pimarenely-15-one, and three known metabolites, ent-8(14)-pimarene-15R,16-diol, stigmasterol and β-sitosterol were isolated from the roots of *Ceriops tagal*. These compounds showed potent non-toxic antifouling activities against larval settlements of *B. albicostatus*[36].

Microorganisms: The advantage of using microorganisms as the source for NPAs is that the compounds can be produced fairly rapidly and in large quantities in bioreactors unlike the invertebrates like sponges, soft corals, etc. wherein large quantities of organisms would have to be collected to gain a small quantity of the compound.

Among bacteria, *Pseudoalteromonas tunicata*, isolated from the surface of a tunicate, showed antifouling activity against *B. amphitrite* and *Ciona intestinalis* larvae[75,76]. They produced at least five compounds that inhibited the settlement or development of a range of surface colonizing species[76,77] while ubiquinone from *Alteromonas* sp.

(isolated from surface of *Halichondria okadai*) inhibited settlement of barnacles[35]. Also, antifouling diketopiperazines have been isolated from a deep-sea bacterium, *Streptomyces fungicidicus*[78].

Bacteria growing on the surfaces of larvae of some crustaceans produce antimicrobial compounds which protect the developing larvae from infections[39,79,80]. Bacteria isolated from the seaweeds have also been shown to release compounds that repel other fouling bacteria, suggesting that they may protect the seaweed from fouling by other organisms[81].

Fungi and cyanobacteria have also yielded NPAs. 3-chloro-2,5-dihydroxybenzyl alcohol (CHBA) from the fungus *Ampelomyces* sp. was detected to have antibacterial and larval settlement inhibition (*B. amphitrite*) properties and cyanobacteria from the cyanobacterium *Scytonema hofmanni* was effective against the fouling benthic diatom *Nitzschia pusilla*[46,82].

NPAs having potential for commercialization

Although numerous NPAs with antisettlement activities have been reported to date, only in a few, but growing number of cases, compounds with potential as replacements for toxic, metal-based antifoulants have been discovered[83] (Figure 4). Some of these NPAs have higher activities compared with those of organotin compounds and copper compounds[15].

Among sponges, polymeric 3-alkylpyridinium salts (poly-APS) isolated from *Reniera sarai* at Consiglio Nazionale delle Ricerche, Italy and Department of Biology, Slovenia, exhibited an EC_{50} of 0.27 μg/ml and LC_{50} of 30 μg/ml against *B. amphitrite* cyprids[61]. On account of its low toxicity, solubility, stability, reversibility and ease in synthesis, poly-APS is portrayed as a promising nontoxic NPA. Poly-APS inhibits acetylcholine esterase enzyme in barnacle cyprids which has a neurotransmitter/neuromodulator role in settlement of barnacle cyprids[61]. 10 *β*-formamidokalihinol A, a terpenoid with a chlorine group attached to its structure, is another potential NPA with EC_{50} of 0.05 μg/ml and LC_{50} > 100 μg/ml indicating its low toxicity towards barnacle cyprids[40]. The NPA agelasine D from the sponges of the genus *Agelas*, together with its two analogues, i.e. AV1003A and AKB695, displayed a strong inhibitory effect on settlement of *B. improvisus* cyprid larvae. Agelasine D had an EC_{50} value of 0.11 μM while the two analogues AV1033A and AKB695 had EC_{50} values of 0.23 and 0.3 μM respectively. None of these three compounds affected larval mortality indicating its potential as an environmentally-compatible NPA[50]. The sesquiterpene hydroquinone, avarol, isolated from *Dysidea avara* at the Department of Pharmacognosy and Chemistry of Natural Products, University of Athens, Greece, has good future prospects on account of the significant anti-settlement activity exhibited by its analogue, 4′-propylthioavarone against cyprids of the barnacle, *B. amphitrite*. It also exhibited a therapeutic ratio of more than 40, highlighting its non-toxic nature[62].

Among soft corals, *Leptogorgia virgulata* and *Renilla reniformis* have been studied intensively for isolating NPAs[23–25,84,85] resulting in the isolation of pukalide with an EC_{50} of 0.05 μg/ml and renillafoulin A with an EC_{50} of 0.02–0.2 μg/ml against *B. amphitrite*. These NPAs also produced encouraging results in the field as well. However, renillafoulins and pukalide are comparatively complex and thus are not amenable for commercial exploitation. Therefore, analogues of pukalide and renillafoulins were synthesized and evaluated for antifouling activity. Among the analogues, khellin exhibited promising antifouling activity and was effective in preliminary field trials[86].

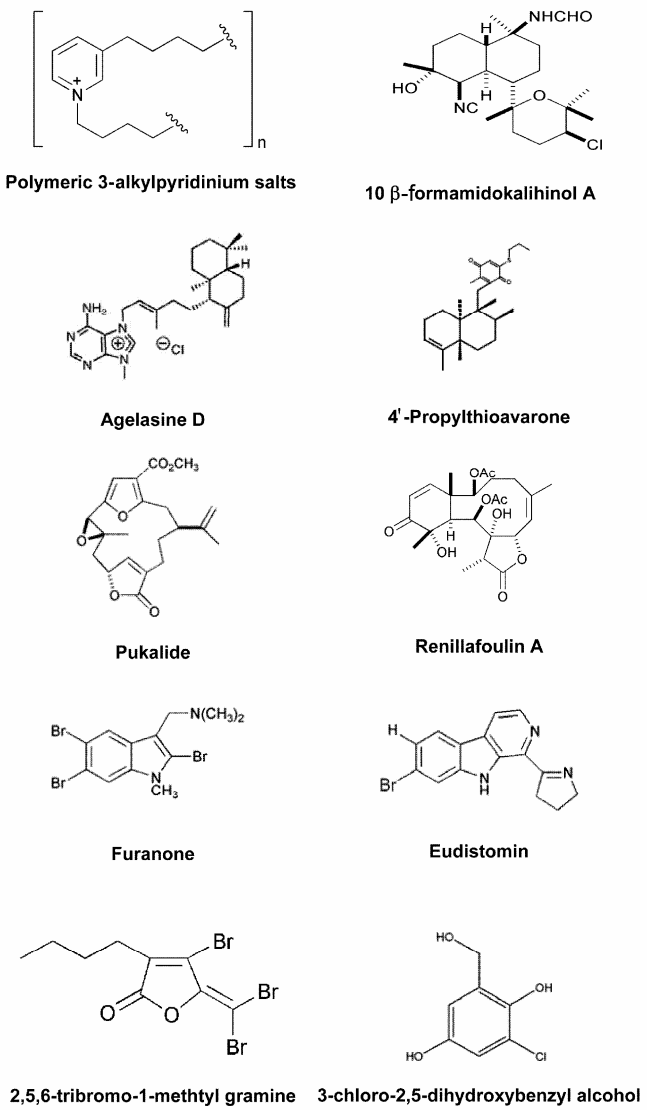

Figure 4. Structures of potential NPAs from marine organisms.

Although there are not many potential NPAs from seaweeds, the most promising NPA, furanone, is from the red seaweed, *Delisea pulchra*. Furanones act by inhibition of the microfouling bacteria. But it remains to be tested whether this automatically prevents barnacle attachment, and if not, how the furanones act against the barnacles.

The most successful NPA isolated from ascidians so far is eudistomins from *Eudistoma olivaceum.* They were found to inhibit larval settlement of *B. neritina* at concentrations[70] less than 2.2 μg/cm^2.

Also, 2,5,6-tribromo-1-methtyl gramine from *Zoobotryon pellucidum*, having remarkable antifouling activity, is projected as potential non-toxic antifoulant with possible scope for commercialization. Its analogue 5,6-dichlorogramine exhibited potent activity against barnacle larvae and mussels (EC_{50} 4 ng/ml)[33,87]. 2,5,6-tribromo-1-methtyl gramine is believed to inhibit serotonergic neurons in barnacle cyprids, which is essential for settlement[88].

Similarly, 3-chloro-2,5-dihydroxybenzyl alcohol isolated from the fungus *Ampelomyces* sp. is also having bright commercial prospects on account of its low toxicity (therapeutic ratio >80) and simple molecular structure[46].

NPA-based paints

Intensive research towards the development of NPA-based paints is progressing worldwide and results seem to be highly encouraging. However, much of these information are trade secrets and hence, not much details are available in a documented form. In general, the available information suggests that NPA-based paints are formulated mainly by using analogues, as the organisms produce NPAs only in trace amounts.

Some of the NPA-based antifouling paints such as 'Sea Nine-211', 'Netsafe' and 'Pearlsafe' have already been commercialized. The NPA in 'Sea Nine-211' paint is 4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one (DCOI), a member of the isothiazolone family. Similarly, 'Netsafe' and 'Pearlsafe' are developed from analogues of furanone isolated from Australian red seaweed, *D. pulchra*[29].

Another antifouling paint being developed consists of 5,6-dichlorogramine, an analogue of 2,5,6-tribromo-1-methylgramine isolated from *Z. pellucidum*. It was coated on the surface of acryl board, and the board was found to be fouling-free even after two months in seawater[15].

Also, reports are available on extracts being directly used in antifouling coatings. For example, extracts of the sea pansy, *R. reniformis*, added to commercially available paint and encapsulated in metallic microtubules[86,89,90] were effective in controlling biofouling over short periods in the marine environment. Paint formulations incorporating extracts of sponges were also active in barnacle settling assays[91]. Apart from this, one paint containing extract of epiphytic bacteria, *Pseudomonas* sp. strain, NUDMB50-11, showed excellent antifouling activity[76].

Major issues in NPAs research

A major issue for NPAs is finding an environmentally responsible way of obtaining adequate supplies of the compounds of interest. Possible ways to overcome this problem include aquaculture, cell culture, analogue development, chemical synthesis, and particularly with regard to bacteria, genetic manipulation and fermentation technology[83,92]. For comparatively simple molecules, chemical synthesis may be the favoured option.

Similarly, the controlled leaching of the natural compounds at desired concentrations after incorporation in antifouling coatings for long-term duration is another challenge confronting the NPAs scientists. Mimicking natural release concentrations by the parent organisms at environmentally safe concentrations may be a tedious task ahead. Method for controlling the release of natural products was investigated by Price *et al*.[89] using microencapsulation technologies. However, more intensive collaborative research efforts involving biologists, organic chemists, polymer and paint technologists are necessary for achieving this goal.

The most widely held view is that the preferred future antifouling system includes a bioactive ingredient, with less non-target toxic effects, and incorporated in a marine paint to deter colonization rather than killing of established foulers.

Conclusion

This review highlights the potential of NPAs as alternatives to TBT-based antifouling coatings. Over 145 NPAs have so far been isolated from various marine organisms. Among these, more than 10 are labelled as potential NPAs owing to their high activity with relatively low toxicity. One of the major problems hampering the development of many promising NPAs is ensuring supply commensurate with the needs of the antifouling paint industry. Unfortunately, NPAs are produced in trace amounts and therefore, relying on source organism may not be a feasible option. Chemical synthesis is the direct way for assuring adequate supply, but most likely, it will be an expensive affair. Another alternative is to develop analogues of NPAs with simple structures that can be synthesized in a cost-effective manner. Of late, more research is focused on the isolation of NPAs from microorganisms, considering the possibility of large scale production, because these organisms are easy to harvest within the laboratory. In spite of all these impediments, the successful commercialization of a few NPA-based antifouling coatings such as Sea Nine-211, Netsafe and Pearlsafe provides optimism.

For every natural cause there is a natural solution. Let us have patience to identify and implement it to protect and preserve Mother Nature for the future generations.

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