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ORIGINAL ARTICLE

Anti-microfouling Activity of Lipidic Metabolites from the Invasive Brown Alga *Sargassum muticum* (Yendo) Fensholt

Erwan Plouguerné · Efstathia Ioannou · Panagiota Georgantea · Constantinos Vagias · Vassilios Roussis · Claire Hellio · Edouard Kraffe · Valérie Stiger-Pouvreau

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Abstract The purification of the chloroform extract from the brown invasive macroalga *Sargassum muticum*, through a series of chromatographic separations, yielded 12 fractions that were tested against strains of bacteria, microalgae, and fungi involved in marine biofilm formation. The chemical composition of four (a, c, g, and k) out of the six fractions that exhibited anti-microfouling activity was investigated. Fraction a contained saturated and unsaturated linear hydrocarbons (C_{12}–C_{27}). Arachidonic acid was identified as the major metabolite in fraction c whereas fraction g contained mainly palmitic, linolenic, and palmitoleic acids. Fraction k was submitted to further purification yielding the fraction kAcaF1e that was composed of galactoglycerolipids, active against the growth of two of the four bacterial strains (*Shewanella putrefaciens* and *Polaribacter irgensii*) and all tested fungi. These promising results, in particular the isolation and the activity of galactoglycerolipids, attest the potential of the huge biomass of *S. muticum* as a source of new environmentally friendly antifouling compounds.

Keywords Antifouling · Biofilms · *Sargassum muticum* · Anti-microfouling · Fatty acids · Galactoglycerolipids

E. Plouguerné (✉) · V. Stiger-Pouvreau
Université Européenne de Bretagne,
Université de Brest, EA LEBHAM 3877,
European Institute for Marine Sciences (IUEM),
Place N. Copernic,
29280 Plouzané, France
e-mail: eplouguerne@hotmail.com

E. Ioannou · P. Georgantea · C. Vagias · V. Roussis
Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens,
Panepistimiopolis Zografou,
157 71 Athens, Greece

C. Hellio
School of Biological Sciences, University of Portsmouth,
Portsmouth PO1 2DY, England

E. Kraffe
UMR CNRS 6521, Université de Bretagne Occidentale,
CS 3837,
Brest 29238, France

Present Address:
E. Plouguerné
Laboratório de Produtos Naturais e Ecologia Química Marinha,
Departamento de Biologia Marinha, Instituto de Biologia,
Universidade Federal Fluminense,
Niterói 24001-970, Brazil

Introduction

Any unprotected surface immersed in water will face an undesirable accumulation of microorganisms, plants, and animals. This phenomenon is called biological fouling or more commonly biofouling (Yebra et al. 2004) and is responsible for many adverse effects especially in the shipping (Beech 1999; Beech and Sunner 2004) and aquaculture enterprises (Braithwaite and McEvoy 2005). Among the different solutions tried throughout the maritime history, tributyltin self-polishing copolymer paints (TBT-SPC paints) have been the most successful in combating biofouling (Yebra et al. 2004). The side effects of TBT were studied thoroughly when antifouling (AF) paints were correlated to the worldwide decline of marine molluscs in coastal areas (Blaber 1970; Smith 1981). The toxicity and environmental impact of the TBT-based paints led to the enforcement of strict laws for the protection of marine and freshwater ecosystems and triggered a scientific race for a more efficient compound (Antizar-Ladislao 2008). As a

consequence, the use of TBT in small boats has been prohibited in many countries since the mid-1980s (Konstantinou and Albanis 2004). In 1982, France banished the use of organotin-based AF paints on boats smaller than 25 m (Alzieu et al. 1986) and shortly after followed North America, UK, Australia, New Zealand, and Hong Kong. The majority of the European countries adopted similar strategies after 1988 (Alzieu et al. 1989; Dowson et al. 1993; De Mora et al. 1995; Champ 2000, 2003). The International Maritime Organization (IMO) called for a global treaty that would ban the application of TBT-based paints from January of 2003, and a total prohibition from 2008 (IMO 2001; CD Commission Directive 2002). As a consequence, the need for development of new environmentally compatible AF technologies is now urgent.

Marine algae, as well as any benthic organisms, are particularly affected by marine biofouling (epibiosis) and it has been proven that they produce secondary metabolites with antibacterial, antialgal, antifungal, antiprotozoan, and anti-macrofouling properties to keep their surfaces free of epibionts (Paul 1992; Hellio et al. 2001, 2002; Steinberg and de Nys 2002; Kubanek et al. 2003; Abarzua and Jakubowski 1995; Abarzua et al. 1999; Etahiri et al. 2001; Hellio et al. 2001, 2002; Bhosale et al. 2002; Da Gama et al. 2002; de Nys and Steinberg 2002; Fusetani 2004; Maréchal et al. 2004; Plouguerné 2006; Plouguerné et al. 2006a; Barbosa et al. 2007; Tsoukatou et al. 2007; Cassano et al. 2008; Culioli et al. 2008; Mokrini et al. 2008). Therefore, natural products from marine algae appear as a promising alternative source of new environmentally friendly AF compounds (Hellio et al. 2009).

Previous studies have proven the value of searching for such activities in algal extracts. As example, Freile-Pelegrin and Morales (2004) demonstrated the activity of 18 out of 21 algal species from the coasts of Yucatan (Mexico) against fouling bacteria. Another study conducted by Hellio et al. (2004) highlighted the potential of macroalgae from Brittany (France) against numerous organisms related to fouling. Recently, Da Gama et al. (2002) reported antifouling activity of several red and brown seaweeds from the Brazilian coast.

In this study, the extract of the invasive alga *Sargassum muticum* from the coast of Brittany, which had showed promising activity earlier (Plouguerné et al. 2008), was further investigated for the isolation of AF compounds. This species is present throughout the year in Brittany with a peak in population density during summertime and the highest level of chemical defense in springtime (Plouguerné et al. 2006b). A previous study, on a number of extracts obtained from *S. muticum*, showed that the chloroform extract was particularly active against several bacterial, fungal, and microalgal strains involved in marine biofilm (Plouguerné et al. 2008). The present study focused on the detection and characterization of antifouling compounds derived from the chloroform extract of this alga. The AF potency of these fractions was tested against four strains of marine bacteria (*Cobetia marina*, *Shewanella putrefaciens*, *Polaribacter irgensii*, and *Vibrio anguillarum*), four strains of marine microalgae (*Pleurochrysis roscoffensis*, *Exanthemachrysis gayraliae*, *Cylindrotheca closterium*, and *Navicula jeffreyii*), and four strains of marine fungi (*Halosphaeriopsis mediosetigera*, *Asteromyces cruciatus*, *Lulworthia uniseptata*, and *Monodictys pelagica*) involved in marine biofilm formation (Culioli et al. 2008).

Materials and Methods

Algal Collection

S. muticum thalli were collected by hand in the intertidal zone at Dellec (48°21′N, 4°34′W) (Plouzané, France) in April 2006. When transferred to the laboratory, the algal biomass was rinsed with distilled water and the thalli were dipped in absolute ethanol for 30 s to eliminate the surface biofilm (Plouguerné et al. 2008). *S. muticum* specimens were then dried at room temperature in the dark for 24 h and subsequently powdered before extraction.

Extraction

The powdered algal tissues were extracted three times for 12 h (992 g dry weight) with 5 l of chloroform for each extraction. The extract was then filtered and the solvent was evaporated under reduced pressure. The obtained 18.631 g of extract were used for the chromatographic separation steps.

Fractionation

Several chromatographic techniques such as vacuum column chromatography (VCC) and high pressure liquid chromatography (HPLC) were used in the separation process, as shown in Fig. 1. At the first step, the extract was fractionated by VCC on silica gel (Kieselgel 60H Merck, Darmstadt, Germany) using an elution gradient of cyclohexane (c-Hex)/ethyl acetate (EtOAc) and EtOAc/methanol (MeOH). Twenty fractions (300 ml each) were collected in a step gradient elution starting with c-Hex 100% and ending with MeOH 100%. Each fraction was checked by thin layer chromatography (TLC) and fractions with similar profile were combined. TLC was performed with Kieselgel 60 F254 aluminum support plates (Merck). Spots were visualized after spraying with 15% H_2SO_4 in MeOH and charring.

As a result, 12 fractions were obtained (a to l) and were tested against the marine bacteria, microalgae, and fungi mentioned above (Fig. 1).

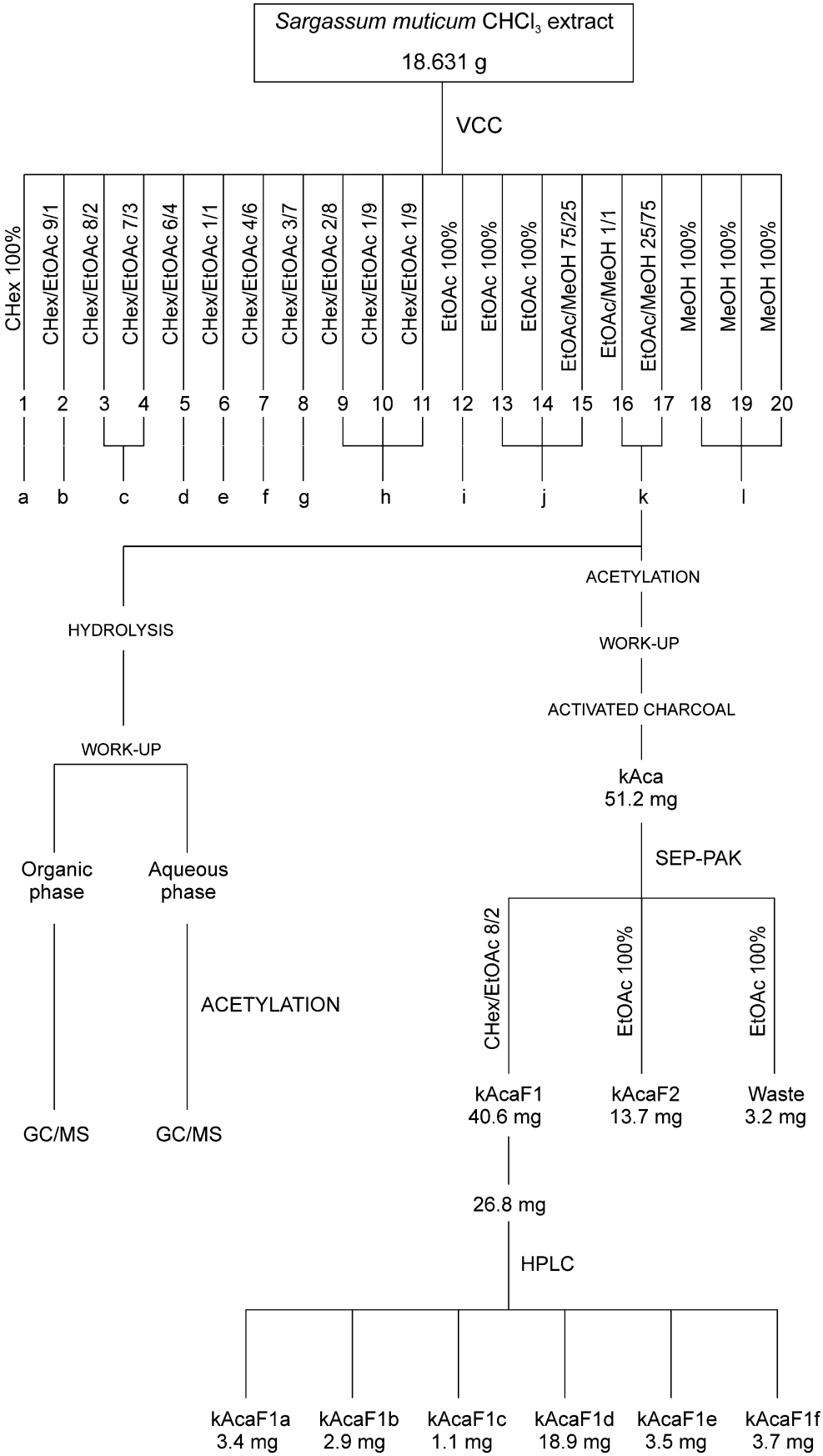

Fig. 1 Purification process of the *Sargassum muticum* chloroform extract

Subsequently, fraction k was filtered through activated charcoal, to remove the chlorophylls, and acetylated with acetic anhydride in pyridine. The work-up of this reaction mixture after drying on $MgSO_4$ was pre-purified on a SEP-PAK® cartridge and three fractions were collected: kAcaF1 (eluted with c-Hex/EtOAc (8/2)); kAcaF2 (eluted with EtOAc 100%), and the waste (Fig. 1).

Fraction kAcaF1 was further purified by HPLC using a Supelco Supelcosil (SPLC-Si, 25 cm×10 mm) column on CECIL 1100 Series Liquid Chromatography Pump connected to a GBC LC-1240 refractive index detector. The mobile phase was c-Hex/EtOAc (8/2) with a 2 ml/min flow rate in isocratic elution. The injection size was 200 µg at a concentration of 1 mg/100 µl.

Structural Analyses

Gas chromatography–mass spectrometry (GC–MS) analyses were carried out using a Hewlett-Packard 5973-6890 GC–MS system operating in electron ionization mode at 70 eV, equipped with a split–splitless injector. Injector was set at 230°C in a split ratio 1:10. The column employed for the analysis was a HP-5 MS fused silica capillary column (30 m× 0.25 mm; film thickness 0.25 µm). The carrier gas was helium at a flow rate of 1 ml/min. The oven temperature was 60°C at the time of the injection and gradually raised to 250°C at a rate of 3°C/min and finally held at 250°C for 10 min. The relative component concentrations were calculated from the total ion counts. Identification of chemical constituents was based on comparison of the Rt values and mass spectra with those in the NIST/NBS and Wiley libraries.

Nuclear magnetic resonance (NMR) spectra were recorded using Bruker DRX 300 and DRX 400 spectrometers and $CDCl_3$ as the solvent. Chemical shifts are given in ppm using TMS as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet).

Chemical Modifications

Fractions a, c, g, and k were methylated prior to their analysis by GC–MS. Five milligrams of each fraction was dissolved in 2 ml of 5% (*v*/*v*) AcOCl in MeOH and kept under continuous stirring at 80°C for 1 h. The reaction mixture was evaporated under vacuum and the residue was partitioned between *n*-hexane and H_2O. The organic phase was dried over anhydrous Na_2SO_4 and kept in hexane for further GC–MS analysis.

Hydrolysis of fraction k was performed in order to analyze the fatty acid moieties of the glycolipids present in this fraction. For this purpose, approximately 5 mg of fraction k was dissolved in 10 ml of 1 M HCl in MeOH and stirred overnight under reflux. The reaction mixture was concentrated and subsequently partitioned between H_2O and CH_2Cl_2. The resulting organic layer was then dried over anhydrous Na_2SO_4 and analyzed by GC–MS for the identification of the methylated fatty acids (Fig. 1).

Acetylation was performed on the residue of the aqueous layer from the work-up of the hydrolysis of fraction k in order to identify the sugar moieties constituting the glycolipids of this fraction. The residue was allowed to react with acetic anhydride in pyridine at 50–70°C with stirring overnight. The work-up of the reaction mixture was realized using CH_2Cl_2/H_2O (1/1). The organic phase containing the acetylated compounds was collected and dried over anhydrous sodium sulfate. After filtration, the organic phase was evaporated to afford 64.2 mg of an oily residue (Fig. 1).

Antibacterial Assays

The chloroform crude extract, the chromatographic fractions and sub-fractions were tested for their inhibitory activity against the growth of four strains of marine bacteria: *Cobetia marina* (ATCC 25374), *Shewanella putrefaciens* (ATCC 8071), *Polaribacter irgensii* (ATCC 700398), and *Vibrio anguillarum* (ATCC 19105). Bacterial strains were maintained on agar plates (LB medium, NaCl (35 g/l), agar (15%)). Experiments were run as previously described by Maréchal et al. (2004). Each treatment and control (seawater) was repeated six times. The crude extract and fractions (at concentrations of 0.75 mg/l) were incubated with the bacteria (2.10^8 cells/ml) in 96-well plates (VWR) in LB medium (Luria Hinton Broth, Sigma, Andover, UK), supplemented with NaCl (35 g/l), at 30°C for 72 h. After incubation, the intensity of growth in presence of the tested compounds and control was compared.

Antifungal Assays

The chloroform extract, the fractions, and sub-fractions were also tested for their inhibitory activity against four strains of marine fungi obtained from the collection of the University of Portsmouth (School of Biological Sciences): *Halosphaeriopsis mediosetigera*, *Asteromyces cruciatus*, *Lulworthia uniseptata*, and *Monodictys pelagica*. The fungal strains were maintained on maize meal agar (Oxoid, Basingstoke, UK) slopes. For the AF assay, the protocol previously described by Hellio et al. (2000) was used: 600 µg of each sample was diluted in 5% DMSO (dimethyl sulfoxide), filtered (Millex-GV unit 0.22 µm Millipore Watford UK pore size), and then incorporated into 6 ml of maize agar 12%, pH 6 (Sigma). Then the Petri dish was inoculated aseptically at the center with an 8-mm-diameter agar plug of mycelium. All assays were done in duplicate and incubated at 25°C for 4 weeks. The growth zones were then recorded and compared with the controls.

Anti-microalgal Assays

The crude extract and the fractions were tested for their inhibitory activity against the benthic phase of four strains of marine microalgae obtained from Algobank (University of Caen-Basse Normandie, France): *Pleurochrysis roscoffensis* (AC32), *Exanthemachrysis gayraliae* (AC15), *Cylindrotheca closterium* (AC170), and *Navicula jeffreyii* (AC181). All microalgal cultures and assays were kept under controlled conditions in a constant temperature chamber at 18±2°C. The photoperiod was 15:9 light:dark (54 µmol photons m^{-2} s^{-1} cool-white fluorescent lamp). Stock of strains was kept on agar plates (F/2, agar 12.5%) (Guillard and Ryther 1962). Experiments were carried out in six replicates and run as previously described in Tsoukatou et al. (2002): 100 µl of a culture at 0.4 µg/ml of chlorophyll *a* were introduced in 96-well plates containing the crude extract or fractions (at a concentration of 0.75 mg/l). After 48 h, the relative optical density of the sample suspension was measured at 600 nm and compared with the controls.

Results

Isolation of Anti-microfouling Fractions

All results concerning the activity of fractions and subfractions of the *S. muticum* chloroform extracts are summarized in Table 1.

Fraction a showed activity against one bacterial strain (*P. irgensii*) and three microalgal strains (*P. roscoffensis*, *E. gayraliae*, and *C. closterium*). Fraction c was found active against three microalgal strains (*P. roscoffensis*, *E. gayraliae*, and *N. jeffreyii*) and fraction g showed activity against three of the bacterial strains (*S. putrefaciens*, *P. irgensii*, and *V. anguillarum*) and all the microalgal strains. Finally, fraction k was active against two bacterial strains (*S. putrefaciens* and *P. irgensii*) and all microalgal and fungal strains tested.

Based on the respective antifouling activity levels, fractions a, c, g, and k were selected for further chemical analyses.

Fraction KAcaF1e, resulting from the purification of fraction k, appears to be active against two bacterial strains (*S. putrefaciens* and *P. irgensii*) and all fungi tested.

Chemical Analysis of Fraction a

Fraction a, which showed activity against one bacterial strain (*P. irgensii*) and three microalgal strains (*P. roscoffensis*, *E. gayraliae*, and *C. closterium*) was analyzed by GC–MS to reveal the presence of saturated and unsaturated linear hydrocarbons (C_{12}–C_{27}) (Table 2). The dominant constituents in this fraction were 1-tetradecene and 1-hexadecene (23.5% and 19%, respectively) (Table 2).

Chemical Analysis of Fraction c

Fraction c was methylated and then subjected to gas chromatography analyses that revealed mainly the presence of arachidonic acid (20:4*n*−6) (61.7%).

Chemical Analysis of Fraction g

The ^{1}H NMR spectrum of fraction g revealed mainly the presence of fatty acids. In order to identify these fatty acids, fraction g was treated with activated charcoal, to remove residual chlorophylls, and subsequently methylated. Fraction g appears to contain mainly palmitic acid (16:0)

Table 1 Antifouling activity evaluation of *Sargassum muticum* fractions

Fractions	Species											
	B1	B2	B3	B4	M1	M2	M3	M4	F1	F2	F3	F4
a			+		+	+	+	−	−	−	−	−
b	−	−	−	−	−		−	−	−	−	−	−
c	−	−	−	−	+	+	−	+	−	−	−	−
d	−	−	−	−	−	−	−	−	−	−	−	−
e	−	−	+	+	−	−	−	−	+	−	+	−
f	−	−	+	−	−	−	−	−	−	−	−	−
g	−	+	+	+	+	+	+	+	+	+	+	+
h	−	+	−	−	−	−	−	−	−	−	−	−
i	+	−	+	−	−	−	−	−	−	−	−	−
j	−	−	+	−	−	−	−	−	−	−	−	−
k	−	+	+	−	+	+	+	+	+	+	+	+
l	−	+	−	+	−	−	−	−	+	+	−	−
kAcaFle	−	+	+	−	−	−	−	−	+	+	+	+

B1: Cobetia marina, *B2: Shewanella putrefaciens*, *B3: Polaribacter irgensii*, *B4: Vibrio anguillarum. M1: Pleurochrysis roscoffensis*, *M2: Exanthemachrysis gayraliae*, *M3: Cylindrotheca closterium*, *M4: Navicula jeffreyii*, *F1: Halosphaeriopsis mediosetigera*, *F2: Asteromyces cruciatus*, *F3: Lulworthia uniseptata*, *F4: Monodictys pelagica.* The activity is represented by (+): presence of activity at 0.75 mg/l and (−): absence of activity at 0.75 mg/l

Table 2 Composition of fraction a

Compound	%
1-Dodecene	9.60
1-Tetradecene	23.50
1-Pentadecene	1.80
n-Pentadecene	2.10
1-Hexadecene	19.00
n-Heptadecane	3.10
1-Octadecene	11.30
(E)-3-Eicosene	6.40
n-Tricosane	1.40
n-Tetracosane	2.00
n-Pentacosane	2.10
n-Hexacosane	1.50
n-Heptacosane	1.20

(33.1%), linolenic acid (18:3*n*−3) (19.5%), and palmitoleic acid (16:1*n*−7) (14.1%) (Table 2).

Chemical Analysis of Fraction k

The ^{1}H NMR spectrum of fraction k revealed the presence of glycolipids from the characteristic signals of the sugar moiety, the glycerol backbone, and the fatty acid chains (Table 2). Following an acid hydrolysis and work-up of the reaction mixture with CH_2Cl_2/H_2O (1/1), the organic and aqueous phases were separated and collected. The aqueous phase containing the sugars was acetylated and then subjected to GC–MS analysis (Fig. 1).

The chromatogram revealed a major constituent that was identified by comparison of its mass spectrum with those of the NIST/Wiley libraries as D-galactopyranoside, methyl, and tetraacetate (58.6%). Analysis of the organic phase by GC–MS allowed the identification of eicosapentaenoic (20:5*n*−3), stearidonic acid (18:4*n*−3), palmitic (16:0), arachidonic (20:4*n*−6), and linolenic (18:3*n*−3) acids as the major fatty acids of the glycolipid esters present in fraction k (Table 3).

Additionally, fraction k was acetylated, treated with activated charcoal, and fractionated on a SEP-PAK cartridge to yield three fractions (kAcaF1, kAcaF2, and waste) (Fig. 1). Fraction kAcaF1 was subsequently purified by HPLC, and six peaks were collected (a–e) and tested against the mentioned microorganisms. Fraction kAcaF1e showed activity against two bacterial strains (*S. putrefaciens* and *P. irgensii*) and all fungi tested at a concentration of 0.75 mg/l (Table 1). ^{1}H NMR spectrum of fraction kAcaF1e showed the characteristic signals of glycolipids. However, in this fraction, characteristic signals of a galactoglycerol moiety were observed (Figs. 2 and 3): galactose moiety (5.35 m, H-4′; 5.18 m, H-2′; 4.98 dd:10.0, 2.5, H-3′; 4.45 d:7.5, H-1′; 4.07 m, H_2-6′; 3.86 m, H-5′) and glycerol moiety (5.19 m, H-2; 4.29 dd:12.0, 4.0, H-1a; 4.12 m, H-1b; 3.93 dd:11.0, 5.0, H-3a, 3.65 dd:11.0, 5.0, H-3b).

Additionally, the MS analysis (with direct insertion probe) performed on fraction kAcaF1e showed characteristic predominant ions at *m*/*z* 169 and 211 indicating a galactose moiety in the molecules constituting the fraction. Analysis of the fatty acids of the glycerogalactolipid highlighted that the purification process had removed galactoglycerolipids containing polyunsaturated fatty acids (PUFAs) from fraction kAcaF1e. Indeed, PUFAs constitute 70.29% of total fatty acids in fraction k in contrast to the 8.02% of the total fatty acids in fraction kAcaF1e (Table 3). The major fatty

Table 3 Composition of fractions k, g, and kAcaF1e

Compound	Fraction k	Fraction kAcaFle	Fraction g
Myristic acid (14:0)	2.54	20.22	7.50
Pentadecanoic acid (15:0)	0.25	2.99	
Palmitic acid (16:0)	15.47	40.01	33.10
Stearic (18:0)	0.66	5.87	
Palmitoleic (16:1*n*−7)	4.04	13.24	14.10
Oleic (18:1*n*−9)	6.48	8.76	
Vaccenic acid (18:1*n*−7)	0.26	0.90	
Hexadecadienoic acid (16:2*n*−4)	1.06	2.08	
Linolenic (18:2*n*−6)	7.90	1.39	6.50
Linolenic (18:3*n*−3)	11.31	2.02	19.50
Stearidonic (18:4*n*−3)	16.13	1.97	
Arachidonic (20:4*n*−6)	1.88	0.00	7.80
Eicosapentaenoic (20:5*n*−3)	17.45	0.56	
Total saturated fatty acids	18.92	69.08	40.60
Total mono-unsaturated fatty acids	10.79	22.90	14.10
Total polyunsaturated fatty acids (PUFAs)	70.29	8.02	33.80

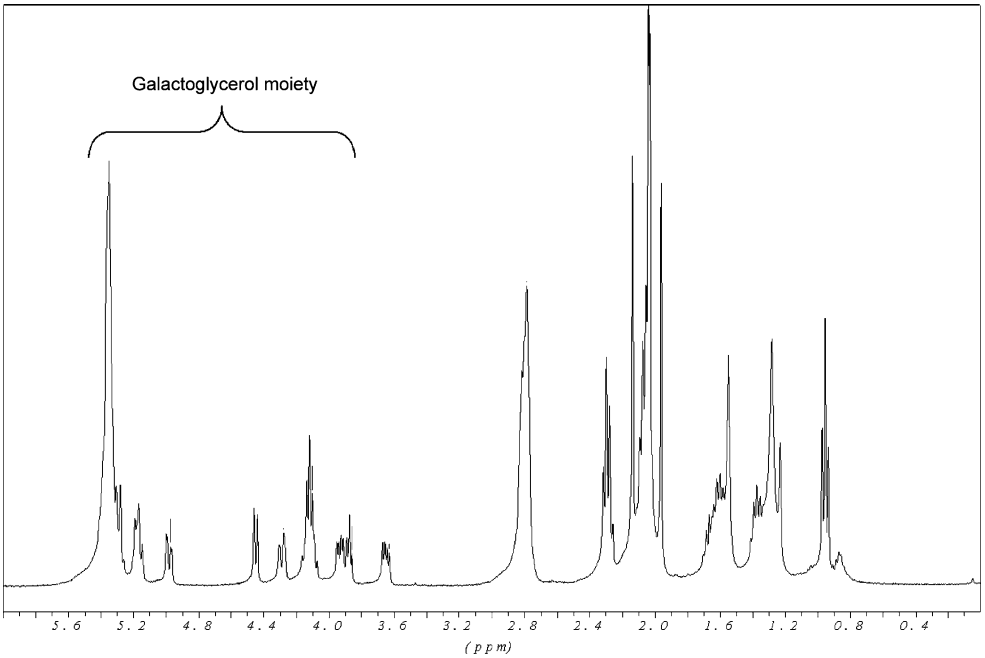

Fig. 2 ^{1}H NMR spectrum of fraction KAcaF1e in CDCL3 (Bruker, 400 MHz)

acids contained in fraction kAcaF1e were identified as 16:0 (40.01%), 14:0 (20.22%), and 16:1*n*−7 (13.24%).

Discussion

The need for new solutions to prevent biofouling has considerably increased since the prohibition in the use of the organotin coatings. New AF products has been developed containing zinc or copper associated with organic biocides such as Irgarol 1051, diuron, and dichlofluanid (commonly used) designed to inhibit algal photosynthesis (Di Landa et al. 2006). Many surveys report the occurrence of such compounds in marine and estuarine environments around the world at alarming concentrations that appear to be toxic to other non-target organisms (Karlsson and Eklund 2004; Konstantinou and Albanis 2004; Koutsaftis and Aoyama 2007). For this reason, diuron is no longer approved as an AF agent in marine paints for use in the UK on any size of vessels

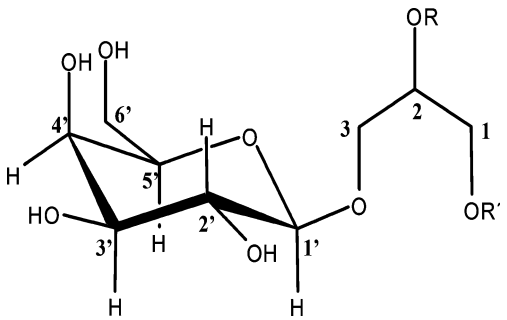

Fig. 3 Structure of galactoglycerol moiety with R and R′: fatty acid esters on the glycerol

(Konstantinou and Albanis 2004). Such studies show the limitation of biocides as new AF agents. Alternatively, soft-bodied sessile marine organisms, and especially macroalgae, represent a very promising source of new environmentally friendly AF compounds.

The majority of the studies reporting AF activity of compounds from brown seaweeds have focused on the bioactivity of phlorotannins isolated from algae as *Fucus vesiculosus* (McLachlan and Craigie 1964), *Fucus spiralis* (Fletcher 1975; Langlois 1975), *Sargassum natans* (Sieburth and Conover 1965), *Sargassum vestitum* (Jennings and Steinberg 1997), *Ascophyllum nodosum* (Langlois 1975; Hellio et al. 2001), *Ralfsia spongiocarpa* (Fletcher 1975), *Pelvetia canaliculata* (Glombitza and Klapperich 1985), *Scytosiphon lomentaria* (Langlois 1975), and *Ecklonia radiata* (Jennings and Steinberg 1997). However, AF compounds other than phlorotannins have been isolated from Phaeophyceae. Indeed, Schmitt et al. (1995) reported the anti-macrofouling activity of the diterpene alcohols dictyol E and pachydictyol A from *Dictyota menstrualis* against *Bugula neritina*. Kubanek et al. (2003) isolated from *Lobophora variegata* the metabolite lobophorolide which exhibits antifungal properties. Barbosa et al. (2007) showed the AF activity of the hexane extract and a dolabellane diterpene isolated from the Brazilian brown alga *Dictyota pfaffii*.

The present study reports the isolation of interesting marine antibacterial, antifungal, and anti-microalgal compounds from *S. muticum*. The identified compounds, polyunsaturated and saturated hydrocarbons as well as the fatty acids and galactoglycerolipids in fractions that exhibited antifouling activity, suggest their potential role

as AF agents. Such results confirm the AF role of lipidic compounds already demonstrated by Rosell and Srivastava (1987), who highlighted the antibacterial activity of unsaturated fatty acids from *Desmarestia ligulata*, and Katsuoka et al. (1990), who isolated galactosyl and sulfoquinovosyl-diacylglycerols from *Costaria costata* and *Undaria pinnatifida* that exhibit anti-macrofouling activity against *Mytilus edulis*. In a more recent investigation, Ganti et al. (2006) demonstrate the AF activity of fats and phtalic acid derivatives isolated from the brown alga *Sargassum confusum*. Nevertheless, the fraction KAcaF1e, containing only galactoglycerolipids, did not inhibit the growth of microalgae. This could have consequences for the use of such compounds as antifoulants.

As reported by Desbois et al. (2009), if the exact way of action of free fatty acids is still unknown, some mechanism such as membrane damages or peroxidative process may be involved regarding the antibacterial activity of these compounds.

The activities of fractions k and kAcaF1e seem to imply their involvement with PUFAs in specific defense mechanisms against microalgae. Indeed, the decrease of PUFAs in fraction kAcaF1e results in a dramatic reduction of the anti-microalgal activity observed in fraction k. Nevertheless, this last observation should be further investigated since the activity observed in fraction k could also be related to other sugar moieties (other than galactose) that occurred in fraction k and that have been removed during the purification process from fraction kAcaF1e.

The question of the ecological role of the compounds we isolated from *S. muticum* may find a beginning of an answer in the invasive character that presents this alga on the coast of French Brittany. Indeed, the production of AF compounds by *S. muticum* may insure a more successful persistence in a new environment, especially if this defense appears more efficient than the ones developed by native seaweeds (Pereira and Da Gama 2008). Nevertheless, our study only used laboratory experiments and principal constituents of the first steps of marine fouling such as bacteria, microalgae, and fungi, and there is no doubt that further experiments involving other kind of microfoulers (protozoans, yeasts), macrofoulers (mussels, barnacles), and in situ experiments will be necessary to better understand the role of the lipidic compounds we isolated from *S. muticum* (Briand 2009). An interesting study by Deal et al. (2003) demonstrated that the deterrent activity of *F. vesiculosus* against herbivores is exerted from the galactolipids rather than the phlorotannins, highlighting the importance of galactolipids in the defense mechanisms against grazers and questioning the role of phlorotannins in the alga. Indeed, methanolic and ethanolic extracts, containing phlorotannins, were not active against microfoulers (Plouguerné et al. 2006b). It would be interesting to carry out the role of galactolipids in the chemical defense of *S. muticum* to confirm or not the involvement of this class of molecules in the defense of this invasive alga. This could show that galactoglycerolipids may play a multiple ecological role in *S. muticum* (Schmitt et al. 1995).

Assuming any industrial application for the AF lipidic compounds isolated from *S. muticum* is quite early. The way that leads to develop a novel AF system based on natural products as new environmentally friendly antifouling solutions is long and many questions such as the toxicity of the compounds, for example, are still to be answered. Concerning the possibility of chemical synthesis, the industrial production of AF lipidic compounds seems possible as the synthesis of such compounds is now mastered (Columbo et al. 2006; Metzger and Bornsheuer 2006). One other severe constraint to the use of natural products in AF technology is the need for large amounts of biological material which may cause a negative ecological impact. In the case of *S. muticum*, such problem does not exist as this species is invasive and may constitute a major threat to marine biodiversity of the coast of French Brittany, as it has already been described by Sanchez et al. (2005) in northern Spain. Indeed, the anti-microfouling activities highlighted in this paper, as well as the very abundant biomass constituted by *S. muticum* on the coast of French Brittany, made of this alga a promising model for the search of new environmentally friendly solutions to the problem of biofouling.

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