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ORIGINAL PAPER

A new tool to detect high viscous exopolymers from microalgae

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Abstract Microalgae are microorganisms often surrounded by a slime layer made of secreted polymeric substances sometimes including polysaccharides. These polysaccharides, weakly described in the literature, can constitute value-added molecules in several industrial areas. The aim of this article is to show that a new tool, the BioFilm *Ring Test*®, can be used to detect viscous microalgal exopolymers. Two red microalgal strains (*Rhodella violacea* and *Porphyridium purpureum*), one cyanobacterium (*Arthrospira platensis*) and their excreted polymeric fractions were studied. *R. violacea* and *P. purpureum* induced a positive response with the BioFilm *Ring Test*® contrary to *A. platensis*. Finally, the understanding of the fractions viscosity involvement in the BRT response was performed by a rheological study.

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Introduction

Microalgal biofilms represent a general term to depict an assembly of species living wherever the surface gets in contact with water [28]. In these photosynthetic structures, whose average thickness is several micrometers, the microalgae are arranged into parallel layers surrounded by extracellular polymeric substances [21].

This matrix plays a part in the protection against external attacks. It also contributes to biofilm structure, keeping water and capture of nutrients or microorganisms by their adhesive properties [32]. Often described as exopolysaccharides, these exopolymeric substances are more complex and diversified as they include proteins and nucleic acids [2]. Moreover, some biofilms can be structured without secretion of exopolysaccharides. Their morphology is different but their strength is not altered [10, 33].

Only a few studies have investigated the structures of exopolymers or cell-bound polymers from microalgae, implied or not in biofilms, such as those produced by *Cyanospira capsulata* [17], *Porphyridium sp.* [19], *Chlorella vulgaris* [24], *Arthrospira platensis* [29], *Dixioniella grisea* [1], *Botryococcus braunii* [15] or *Rhodella violacea* [5]. In all cases, polysaccharides or proteoglycans have been identified and some of them found industrial applications as those produced by *Porphyridium* species [13, 34]. So, in a context where microalgae are increasingly used in industrial applications, it is important to find a new tool able to detect the ability of microalgae to excrete biopolymers such as exopolysaccharides. This new device is the BioFilm *Ring Test*® whose principle is based on

magnetization of magnetic beads in culture media of microorganisms. If beads are free in culture media, a spot can be observed at the center of the well after magnetization. On the other case, no spot appears because of the immobilization of beads by an extracellular matrix or an increase of viscosity. This kit is so far developed for bacterial screening only. Therefore, the aim of this study is to employ the BioFilm *Ring Test*® (BRT®) to detect exopolymeric substances production by microalgae. Two red microalgae (*Porphyridium purpureum*, *Rhodella violacea)* and a cyanobacterium (*Arthrospira platensis*) previously described for exopolymeric substances production have been chosen as models. Our works show that the BioFilm *Ring Test*® is a powerful tool to screen microalgae able to increase the viscosity of their culture media by production of high molecular weights polysaccharides.

Materials and methods

The BioFilm *Ring Test*®

The principle of this kit was detailed by Chavant et al. [6]. It is performed to determine if microorganisms are able to form a biofilm. These microorganisms are cultivated in microwells of a microplate (96 wells) with magnetic particles. A microplate is divided into 12 strips, and one strip is here used for each incubation time tested. For the reading, wells are first covered with a few drops of Contrast Liquid (inert and non-toxic oil) and scanned with the Plate Reader, a dedicated scanner apparatus, to get an I_0 image. Then, the strip is placed for 1 min on the Block Test and scanned again to get an I_1 image. The Block Test is made of 96 magnets and designed so that each magnet is centered under the bottom of each well. After magnet contact, free beads are attracted in the center of the bottom of wells, forming a spot, while the beads blocked by the biofilm remain in place. Images of each well before (I_0) and after (I_1) magnetization are compared with the BioFilm Control® software, which uses an algorithm to estimate the discrepancy between the two images of the same well, giving a value named the BioFilm Indice (BFI) ranging from 0 to 30. A high BFI value corresponds to a high mobility of beads under magnet action (i.e., control wells), while a low value corresponds to a full immobilization of beads (no differences for a well between its images I_0 and I_1).

Microalgal strains and growth conditions

Arthrospira platensis PCC 8005 was grown in Zarrouk medium modified by Cogne et al. [8]. *Rhodella violacea* was isolated at the Laboratoire "Microorganismes: Génome et Environnement" (LMGE) from a natural sample. It was grown on derived Jones medium modified according to Doan et al. [11, 22]. Additional components issued from Conway medium, a modified version of Walne medium, were added [31]. They were, for 1 l of the derived Jones medium, 100 mg $NaNO_3$; 26 mg $NaH_2PO_4{-}2H_2O$; 100 mg Na_2SiO_3, 1 µl of trace metal solution. Trace metal solution was composed as follows: 0.45 g $(NH_4)_6Mo_7O_{24}{-}4H_2O$, 1 g $CuSO_4{-}5H_2O$ and 50 ml of distilled water.

Porphyridium purpureum CCAP 1380/1A was grown in Artificial Sea Water (ASW) composed by 3.75 ml of extra salts solution (30 g $NaNO_3$, 1.2 g Na_2HPO_4 and 1 g K_2HPO_4 per liter); 2.65 ml of a vitamin solution (0.2 mg biotin, 20 mg calcium pantothenate, 4 mg cyanocobalamin, 0.4 mg folic acid, 1 g inositol, 20 g nicotinic acid, 0.1 g thiamine HCl and 0.6 g thymine per liter); 25 ml of soil extract; 0.5 g of tricine diluted in water qsp 1 l. All cultures are inoculated at 10% (v/v) of the total volume and incubated without shaking at 22°C under strip lighting (18 W) using a photoperiod of 16 h followed by a dark phase of 8 h.

Evaluation of microalgal biofilms with the BioFilm *Ring Test*®

Tests were made with non-inoculated culture media (Zarrouk modified, ASW, Jones medium modified) in the presence or not of Tween 20, added at 0.002% (w/v) in final concentration. The toner was added in each culture media to get a final concentration of 10 µl ml^{-1} and 200 µl per well were loaded. Plates were incubated at 22°C. After 0, 24, 48, 72, 96, and/or 168 h, strips were analyzed with the BFC *Elements*® as described previously.

Arthrospira platensis, *Rhodella violacea* and *Porphyridium purpureum* initial microalgal suspensions (IMS) were diluted until obtaining a clear spot at T_0 with the BioFilm *Ring Test*®. Two wells per strip composed of culture media with magnetic beads and without microorganisms were used as controls. Plates were incubated at 22°C under light as described above and strips were analyzed at different times of incubation with the BFC Elements® as explained previously.

Growth of Arthrospira platensis was followed from wells loaded without toner and incubated in the same conditions by measure of A_{600}. This was not possible for the two red algae due to high level of large aggregates formation.

Production and extraction of exocellular fractions

Microalgae were cultivated in 500-ml flasks containing 200 ml of culture media under artificial light as described above between 6 and 10 weeks before extracellular

fraction extraction. All flasks were inoculated at 10% (v/v) and incubated at 22°C without shaking.

Rhodella violacea and *Porphyridium purpureum* cultures were centrifuged at 10,000 × *g* for 20 min at room temperature to eliminate biomass (a dilution of *P. purpureum* culture was necessary to reduce the too high viscosity before the centrifugation), while the culture of *Arthrospira platensis* was filtrated through a glass filter (10–16 µm).

Supernatant or filtrates were concentrated by evaporation (Büchi RII) under 72 mbar, 60°C, and dialyzed (10,000 Da) against distilled water at 4°C for 48 h. Finally, the products were dried under vacuum and exopolymers were stored at room temperature.

Test of exopolymers with the BioFilm *Ring Test*®

Different concentrations of exopolymeric fractions were tested with the BRT® after their solubilization in specific culture media of their microalgae producer.

Toner was added in each solution at 10 µl ml^{-1}. Plate reading was carried out instantaneously. Controls were composed by media and toner without any exocellular fraction.

Quantitative analyses

Protein concentrations were determined by the Bradford method [3] using BioRad reagent and bovine serum albumin (BSA) as standard.

Total organic nitrogen was measured after a digestion procedure with sulfuric acid and hydrogen peroxide followed by colorimetric assay as developed by Hach [20].

Concentrations of acidic and neutral monosaccharides were determined by colorimetric assays. Uronic acids were quantified by absorbance at 520 nm (A_{520}) after reaction with m-hydroxydiphenyl [4] and neutral sugars by A_{450} after addition of resorcinol in the presence of sulfuric acid [12, 23]. The results were expressed in equivalent of D-glucose for neutral sugars and D-glucuronic acid for acidic sugars.

SEC–MALLS analyses

Average molecular weights and molecular weight distributions were determined by high-pressure size exclusion chromatography (HPSEC) with on line multi-angle laser light scattering (MALLS) filled with a K5 cell (50 µl) and two detectors: a He–Ne laser ($\lambda = 690$ nm) and a differential refractive index (DRI). Columns [OHPAK SB-G guard column, OHPAK SB806, 804 and 803 HQ columns (Shodex)] were eluted with $NaNO_3$ 0.1 M at 0.7 $ml.min^{-1}$. Solvent was filtered through 0.1-µm filter, degassed and filtered through a 0.45-µm filter upstream column. The sample was injected through a 100-µl full loop. The collected data were analyzed using the Astra 4.50 software package.

Spectroscopy

Dried exopolymer samples (3 mg) were dispersed in 100 mg of anhydrous KBr and pressed. The IR spectra were recorded at room temperature in the wave number range of 400–4,000 cm^{-1} and referenced against air with a Nicolet 380 FT-IR instrument (Thermoelectron Corporation). A total of 50 scans were averaged for each sample at 4 cm^{-1} resolution.

Rheological measurements

Rheological measurements were performed with double concentric cylinders geometry on a stress-controlled Rheometer AR-G2 (TA Instrument) with a Peltier temperature control system. Temperature was controlled at 25°C and the viscosity was evaluated depending on the shear rate comprised between 10^{-3} and $10^{3}s^{-1}$. Extracellular fractions were dissolved in pure water at concentrations of 1.5 g l^{-1}, 0.8 g l^{-1} and 0.5 g l^{-1} by gentle stirring at 40°C. Finally, 13 ml of solution was inserted in the instrument. All data were analyzed by the Rheology Advance software.

The Cross equation (1) is a suitable rheological model classically used for such entangled polymer solution [9].

$$\frac{(\eta - \eta_\infty)}{(\eta_0 - \eta_\infty)} = \frac{1}{(1 + (K\dot{\gamma})^m)} \quad (1)$$

η_o is the Newtonian viscosity at low shear rates, η_∞ the Newtonian viscosity at high shear rates, K the consistency (s) and m the rate index.

Results and discussion

Adaptation of microalgae culture media to the BioFilm *Ring Test*®

The BioFilm *Ring Test*® is functional with bacterial-rich media such as Brain Heart Infusion (BHI), Luria–Bertani (LB), and Trypton Soy Broth with Yeast Extract (TSBYE). So, the first step of these experiments was to test the non-inoculated microalgae media mainly composed of mineral and salts, which can increase electrostatic forces that interact with the charged magnetic particles. In bacterial-rich media, the organic particles as proteins and peptides permit to counteract these interactions avoiding the agglomeration of toner in a side of the well (consequently,

magnetic beads are immobilized in the bottom of the wells and cannot gather in the center). To obtain a clear spot at T0 and all along the time of incubation for the controls (media + toner) a BFI >15 was necessary. A BFI below 2 indicated a total immobilization of toner. As shown in Fig. 1, results obtained with non-inoculated media were not satisfying. The addition of a dispersant agent appeared as a solution and Tween 20 (polysorbate 20) was tested as it was a non-toxic and non-ionic molecule able to decrease the surface tension. Experiments were performed for 168 h with several concentrations of Tween. Addition of Tween 20 at 0.002 or 0.005% respect the criteria of BFI >15 cited previously. There was no significant BFI variations along the time contrary to results obtained with Tween 20 at 0.001% (data not shown). The lowest concentration, 0.002%, was retained to be added to the culture media.

Evaluation of exopolymer production with the BioFilm *Ring Test*®

Among the three microalgae species, *Rhodella* sp. and *Porphyridium* sp. were well depicted as viscous exopolysaccharides producers [14, 18], although, there was an ambivalence concerning the abundant literature describing exopolymers from *Arthrospira platensis* [16, 29]. Effectively, no increase of apparent viscosity of culture media was detected after incubation of *Arthrospira platensis* PCC 8005 during 168 h.

When *Arthrospira platensis* was grown in microplates, it was obvious that a spot remained present in the bottom of wells after 168 h of incubation, meaning that *Arthrospira platensis* was not able to adhere on a surface or to increase the viscosity in the Zarrouk medium (Fig. 2). Note that the increase of BFI in the first hours of culture was due to beads sedimentation, the first BFI measurement being immediate after inoculation. They sedimented during the following hours changing the contrast of the image I_0 for the next measurements. As seen by the evaluation of absorbance at 600 nm, the lack of adhesion of *Arthrospira platensis* was not due to an absence of growth. Although a decrease of a BFI was observed but it has remained above two indicating that it was not due to the cyanobacterium adhesion but to the development of the green chlorophylls in relation to *Arthrospira platensis* growth. The color toned down the contrast that explained why BFI decreased (contrast is an important parameter; the BFI being calculated by a difference of images). Consequently, *Arthrospira platensis* was not able to immobilize magnetic particles and constituted a negative for the next experiments.

As explained in the Materials and methods section, it was necessary to inoculate wells with specific media in conditions leading to a non-spot formation (BFI >10). For *Arthrospira platensis* it was possible to use the absorbance at 600 nm as a way of biomass evaluation. For *Rhodella* and *Porphyridium* (well known to form aggregates associated with high viscosity), dilution of the preculture was necessary until the detection of a clear spot at T_0. For *Rhodella violacea*, dilution was 1/100 while it was 1/10 to obtain *Porphyridium purpureum* initial microalgal suspension (IMS). After 48 h of incubation of *Rhodella violacea* culture (diluted at 1/100), BFI value was two as shown in Fig. 3. This BFI was attributed to biopolymers synthesis and/or microalgae proliferation in regard to their generation times described between 3.5 and 24 h [7]. Figure 4 illustrates the behavior of *P. purpureum* in microwells, the BFI has fallen under 2 after 60 h. As for *R. violacea*, the decrease of BFI detected in a few hours was associated with exopolymer production and/or growth. The growths of the two red microalgae couldn't be measured by spectrophotometry as for *A. platensis* but have been qualitatively observed in wells after 100 h of incubation by the development of a red color as seen in Fig. 3.

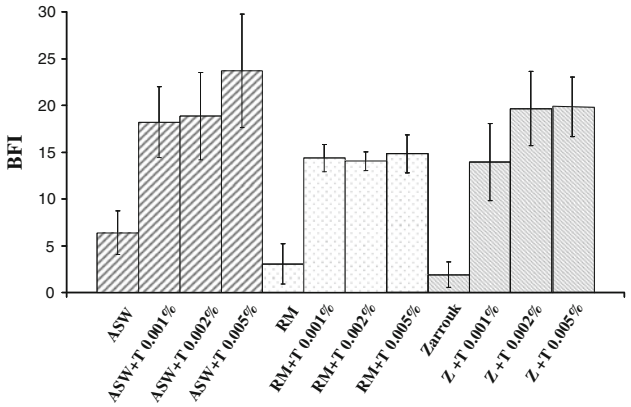

Fig. 1 Tests of media with the BioFilm *Ring Test*®. *ASW* artificial sea water, *RM Rhodella* medium, *Z* Zarrouk modified medium, *T* Tween 20. All experiments were performed in triplicate

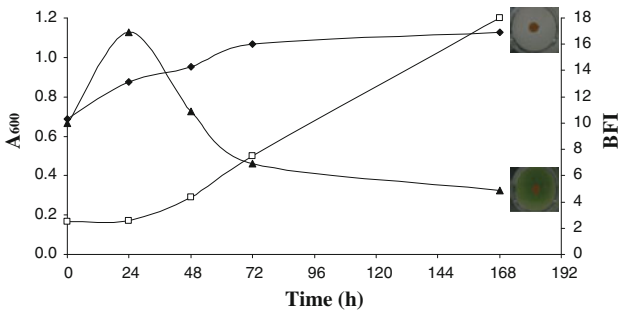

Fig. 2 Growth (*square*) of *Arthrospira platensis* incubated in Zarrouk medium during 168 h at 22°C and biofilm formation (*filled triangle*), (*filled diamond*) Control (Zarrouk medium non-inoculated)

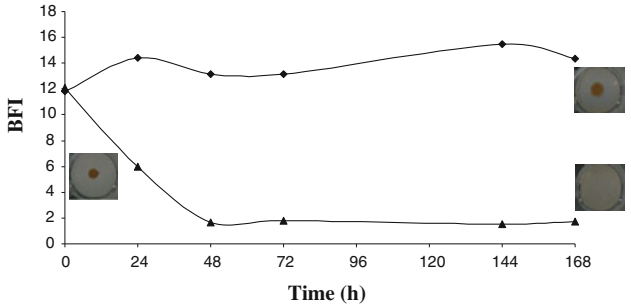

Fig. 3 Biofilm formation by *Rhodella violacea* (*filled triangle*), (*filled diamond*) Control (*Rhodella* medium non inoculated)

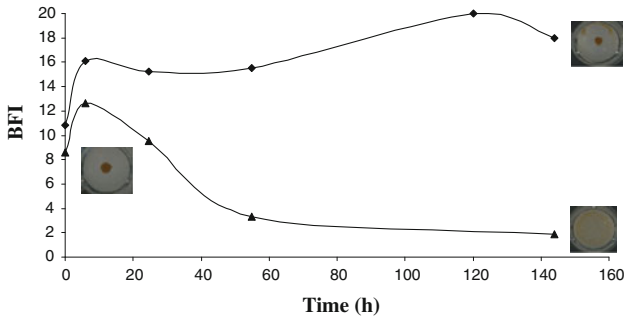

Fig. 4 Biofilm formation by *Porphyridium purpureum* (*filled triangle*), (*filled diamond*) control (ASW non-inoculated)

Implication of exopolymers into BFI

To evaluate the role of putative exopolymers in BFI values, the three microalgae were grown in their specific culture media and their exocellular fractions were extracted. Yields of polymers were 0.12, 0.26, and 0.42 g l^{-1} for *R. violacea*, *P. purpureum* and *A. platensis*, respectively (Table 1). Even if yields are depending not only on culture duration but also on light intensity and light/dark phases, it was possible to correlate them with bibliographic data. Considering *A. platensis*, our results agree with a yield of 0.21 g l^{-1} after 21 days, as published by Trabelsi et al. [29]. From *Porphyridium* sp. cultures, yields were comprised between 0.13 and 1.3 g l^{-1} [25–27, 30]. Our yield of 0.26 g l^{-1} was also consistent. A polysaccharide production from *Rhodella reticulata* attaining 1.63 g l^{-1} when its culture medium was added by nitrates [35] was obviously higher than our yield of 0.12 g l^{-1}. However, our experiences showed a co-precipitation of salts which may induce some overvalued yields when alcoholic precipitation was employed to extract exopolymers. That was the reason why our samples were concentrated and dialyzed but not precipitated by polar solvents such as ethanol or isopropanol.

These dialyzed fractions (with a 10-kDa NMWCO membrane) were tested for their capacity to immobilize the magnetic particles of the BRT (Fig. 5). To make a correlation with previous data, each polymer was dissolved in its specific culture media. Ranges of concentrations were chosen between 0 and 0.8 g l^{-1} because they corresponded to concentrations of the dried and dialyzed fractions detected in culture media after extraction. An important decrease of BFI was noticed (≤ 2) between 0 and 0.2 g l^{-1} of exocellular fractions from red microalgae. Magnetic particles were totally immobilized at 0.2 g l^{-1} for *R. violacea* and 0.4 g l^{-1} for *P. purpureum*. These concentrations were in agreement with the yields of exopolymer extractions, which were respectively of 0.12 g l^{-1} and 0.26 g l^{-1} for *R. violacea* and *P. purpureum*. As expected, the exopolymeric fraction from *A. platensis* did not induce beads immobilization even for higher concentrations (0.8 g l^{-1}) than those present in culture medium. So, chemical and physico-chemical investigations were started to explain this result.

Chemical and physico-chemical characterizations of exopolymeric fractions

To understand the behavior of extracted and dialyzed exocellular fractions, their nature was elucidated by colorimetric assays (Table 1).

Proteins content of exopolymers extracted from supernatant of R violacea was not determined because of precipitation phenomenon between exopolymer solution and Coomassie Blue reagent. However, the very low content of nitrogen of this fraction confirmed its non-proteic nature. The three exopolymeric extracts were mainly composed of neutral sugars and the presence of uronic acids and proteins was evaluated as not significant. The low neutral sugar content could be explained either by the presence of high levels of no sugar components either assuming that the

Table 1 Composition of sugars and nitrogen according to microalgal fractions

Microorganisms	*Arthrospira platensis*	*Rhodella violacea*	*Porphyridium purpureum*
Yields (g l^{-1})	0.42	0.12	0.26
Proteins (g g^{-1})	0.06	–	0.02
Nitrogen (g g^{-1})	0.04	0.01	0.01
Uronic acids (g g^{-1})	0.09	0.07	0.08
Neutral monosaccharides (g g^{-1})	0.39	0.41	0.48

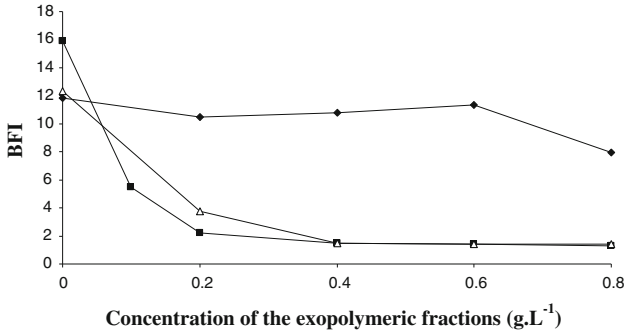

Fig. 5 Biofilm formation by exopolymeric fractions extracted from *Porphyridium purpureum* (*triangle*), *Rhodella violacea* (*filled square*) and *Arthrospira platensis* (*filled diamond*)

absorption coefficient of glucose was inferior to this(ese) of constitutive monosaccharide(s) of these exopolymers. Our results verified the presence of carbohydrates in the different fractions depicted in literature as either polysaccharides [14, 18, 19, 29] or proteoglycan by some strains of *Rhodella* [5]. *A. platensis* polysaccharidic fraction is depicted as a very complex structure of nine monosaccharides (Gal, Xyl, Glc, Fuc, Rha, Ara, Man, GalA, GlcA) by Trabelsi et al. [31]. *Rhodella grisea* is able to produce a heteropolymer of neutral sugars (Gal, Xyl, Glc, Rha, Ara, Man) with a smaller proportion of uronic acids [5]. A structure of *Porphyridium* sp. polysaccharide presents four main sugars (Xyl, Glc, Gal, and GlcA) sometimes with presence of mannose and arabinose [13, 19]. To reinforce this polysaccharidic nature, FTIR analysis was performed on the three products (Fig. 6). Infrared spectra of three samples showed an intense band at 3,400 cm^{-1} which was characteristic of stretching vibration of hydroxyls from monosaccharidic residues of polymer and residual water. The less intensive band detected between 3,000 and 1,850 cm^{-1} was assigned to stretching vibration of C–H. The FTIR spectra displayed an intense signal of absorption detected around 1,650 cm^{-1} and specific of vibrations of symmetric angular deformations from water hydroxyls and asymmetric vibration of C=O. Other bands of low intensity between 1,450 and 1,350 cm^{-1} were specific to symmetric angular deformations of C–C–H. Note the absorption band at 1,250–1,255 cm^{-1} only detected on the spectra of polysaccharide extracted from *P. purpureum* and *R. violacea* media. It was indicative of S=O stretching vibration [18]. Finally, the intensive absorption identified in all spectra at 1,050 cm^{-1} was attributed to C–O and C–OH stretching vibration. All these signals reinforced the polysaccharidic nature sulphated or not of the three exopolymers.

The relatively close chemical composition of the three exopolymers associated with their polysaccharidic nature macromolecules was not sufficient to explain their different behaviors with magnetic particles of the BioFilm *Ring Test*®. Therefore, to validate their polymeric structures, SEC–MALLS analysis was done to appreciate their molecular weights and their polydispersities.

The concentration of polysaccharide macromolecules in the samples was 42% for *R. violacea*, 19% for *P. purpureum*, and 32% for *A. platensis*. This low carbohydrate content led to expect a non-negligible part of salts associated with exopolymers. The relative percentage of each macromolecular family was expressed on behalf of total macromolecules identified for each microalgae (Table 2). Curiously, three families of high-molecular-weight polymers were significantly detected in the extract from *A. platensis* and one of them had the highest molecular weight of all polymers detected in the three microalgal extracts. The two red algae extracts contained polysaccharides with molecular

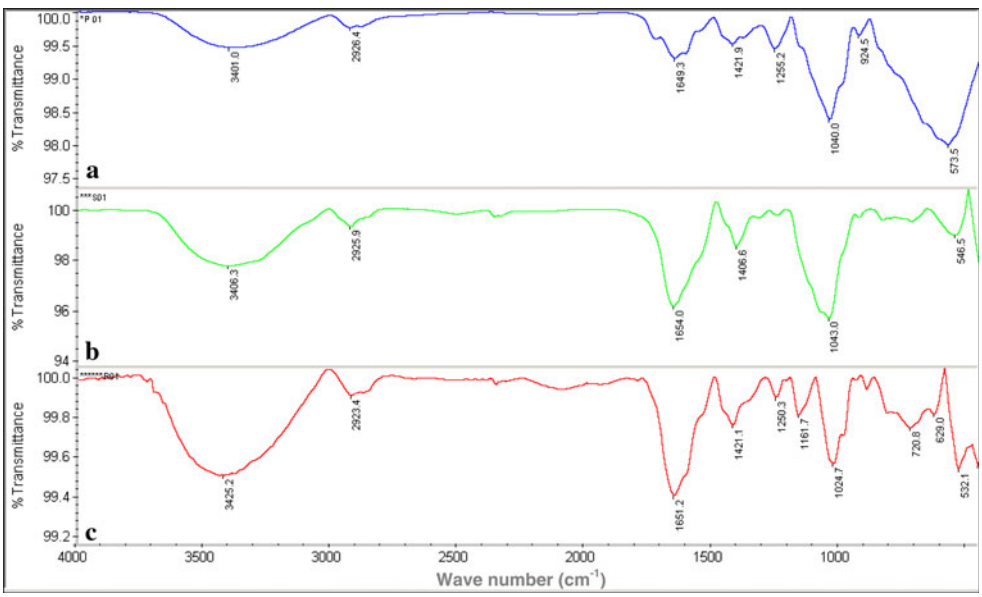

Fig. 6 FTIR analyses of exopolymers extracted from *P. purpureum* (**a**), *A. platensis* (**b**) and *R. violacea* (**c**)

Table 2 Relative distribution of molecular weights in microalgae fractions

		Peak 1	Peak 2	Peak 3	Peak 4
R. violacea	Mw (g mol^{-1})	2.63 × 10^6	–	–	–
	Relative %	100	–	–	–
	Ip	1.005	–	–	–
P. purpureum	Mw (g mol^{-1})	4.07 × 10^6	3.78 × 10^6	3.13 × 10^6	2.14 × 10^6
	Relative %	26	26	32	16
	Ip	1.002	1.001	1.007	1.029
A. platensis	Mw (g mol^{-1})	1.08 × 10^7	7.42 × 10^5	1.17 × 10^6	–
	Relative %	34	50	16	–
	Ip	1.48	1.24	1.06	–

Ip index of polydispersity

Table 3 Cross model parameters

Concentrations	Microalgae	*K* (s)	η_0 (Pa.s)	*m*
1.5 g l^{-1}	*A. platensis*	603.3	0.84	1.14
	R. violacea	52.75	15.10	0.93
	P. purpureum	126.1	18.27	0.83
0.8 g l^{-1}	*A. platensis*	48.46	0.25	1.08
	R. violacea	34.39	2.39	0.85
	P. purpureum	129.5	7.64	0.85
0.5 g l^{-1}	*A. platensis*	37.52	0.07	1.01
	R. violacea	14.71	0.48	0.80
	P. purpureum	178.4	3.81	0.80

K consistency, η_0 viscosity measured at the lowest shear rate, *m* rate index

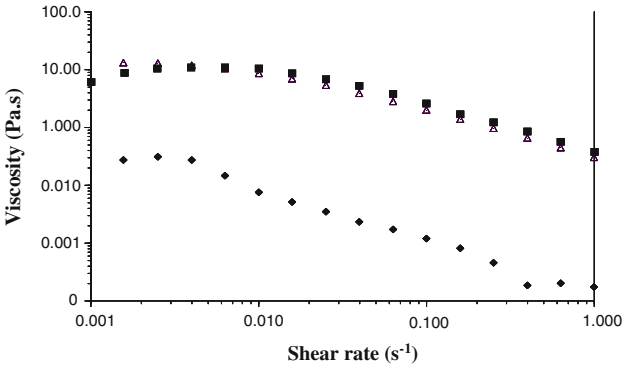

Fig. 7 Viscosity of exopolymeric fractions (1.5 g l^{-1}), (*triangle*) *Porphyridium purpureum*; (*filled diamond*) *Arthrospira platensis*; (*filled square*) *Rhodella violacea*

weights of 2.3 × 10^6 g mol^{-1} for *R. violacea* and between 4.07 and 2.14 × 10^6 g mol^{-1} for *P. purpureum*. Note the heterogeneity of *P. purpureum* and *A. platensis* extracts which contained, respectively, four and three exopolymers families by comparison to *R. violacea* producing only one exopolymer. In order to progress in the understanding of interactions between exopolymers and magnetic particles of the BioFilm *Ring Test*®, rheological investigations were conducted. The aim of this study was to prove that micro-viscosities created in the well by exopolymers were at the origin of BFI decreases.

As a consequence, some preliminary rheological measurements in the ranges of native concentrations (Table 3) of polysaccharide in distilled water were conducted. Flow properties of the more significant concentrations are reported in Fig. 7. No significant differences appeared between flows of the polymers extracted from *R. violacea* and *P. purpureum* whereas strong differences were detected between those extracted from *A. platensis* and those coming from red microalgae. The Cross model applied to these results (Table 3) indicates that the 'zero shear' viscosity (i.e., η_0) of polysaccharides from *A. platensis* was inferior to these of the two red microalgae in water with a factor of 20 for the 1.5 g l^{-1} concentration. Even if this difference decreased for lower concentrations, it remained significant showing that the BFI decrease was in correlation with the increase of viscosity in culture media of red microalgae. Interestingly, the presence of non viscous high-molecular-weight exopolymers as these produced by the strain of *A. platensis* used in this study was not sufficient to obtain BFI <2.

Conclusions

In conclusion, the use the BioFilm *Ring Test*® with microalgae and cyanobacteria was validated after the addition of 0.002% Tween 20 in their culture media. Two red microalgae (*R. violacea* and *P. purpureum*) were able to immobilize particles of toner, inducing a positive response, while the third photosynthetic microorganism was diagnosed as negative. Production of exopolymers has been detected in culture media of the three microalgae. Two of them exhibited significant levels of viscous exopolymers.

These red microalgal polymers have been identified as the agents able to immobilize magnetic particles of the BRT at concentrations equivalent to those expressed by microorganisms. This allows concluding that the BRT can be used as a new tool to detect high viscous exopolymers from microalgal cultures. The role of these macromolecules in biofilm formation will be investigated in the future after screening of enzymes efficient for their degradations. At a time when the number of publications relative to the culture of microalgae for biofuel production has increased significantly, the BRT® could be an interesting tool for screening photosynthetic microorganisms able to generate innovative high viscous exopolymers for industrial applications.

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