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## Screening of marine microalgae: Investigation of new exopolysaccharide producers

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1           **Screening of marine microalgae: investigation of new**  
2                                   **exopolysaccharide producers**

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17

18 **Abstract:**

19 Biopolymers, such as exopolysaccharides are widely exploited by industry as hydrocolloids  
20 (gelling, thickening agents) and biological agents (anti-inflammatory, anti-parasitic,  
21 antioxidant, etc.). In this study, 166 marine microalgae and cyanobacteria species have been  
22 screened in order to identify strains producing original exopolysaccharides. This screening  
23 allowed the highlighting of 45 positive strains. In a second time, the monosaccharide  
24 compositions from 20 EPS of them were determined by GC/MS and HPAEC-PAD. The results  
25 led to a discovery of 8 new genera of microalgae producing EPS, including polymers with a  
26 very original composition like richness in GlcA. Finally, a phylogenic tree has been constructed  
27 in order to assess the link between the phylogeny of microalgae and the global composition of  
28 their exopolymers, based on data obtained in this study and from the literature.

29

30 **Keywords:** Screening, Microalgae, Polysaccharides, Exopolysaccharides, Phylogeny

## 31 **1. Introduction**

32 Microalgae and cyanobacteria are single-cell photoautotrophic microorganisms which  
33 can produce biomass with a great efficiency using solar energy, water and CO<sub>2</sub>. These  
34 organisms represent a huge diversified group including theoretically between 200 000 and 800  
35 000 different species, 35 000 of them being really identified in marine and fresh water  
36 environments but also acidic lakes, hypersaline waters, or desert areas [1]. These photosynthetic  
37 microorganisms can be divided into blue-green algae or cyanobacteria and major eukaryotic  
38 groups including green microalgae (Chlorophyta and Charophyta), red microalgae  
39 (Rhodophyta) and brown-golden microalgae (Ochrophyta, Haptophyta and Miozoa). This large  
40 taxonomic diversity makes microalgae very attractive to produce valuable and diverse  
41 biomolecules such as pigments, proteins including some enzymes, PolyUnsaturated Fatty Acids  
42 (PUFAs), lipids, and exopolysaccharides (EPS). These compounds present some interests for  
43 many diverse industrial areas, like pharmaceutical, nutraceutical, cosmetic and food/feed  
44 production [2-3]. Moreover, the development and commercialization of these metabolites is  
45 still early and only niche markets are currently available for microalgae products. Their low  
46 usage is very surprising but may be easily explained by the costs involved in microalgae  
47 production, limiting their applications to the field of high value products. These costs are linked  
48 to the photoproduction, the harvest of microalgae in diluted media, the recycling of culture  
49 media and the difficulty to refine this biomass [4-5].

50 Among the high value compounds from microalgae, EPS are very promising. Even if  
51 polysaccharides from animals, fungi and higher plants (including macroalgae) have been  
52 studied for many years, interest of the scientific community for EPS produced by microalgae  
53 and cyanobacteria is much more recent as described by Rossi and De Philippis [6] and Delattre  
54 et al. [7]. Based on their function, polysaccharides from photosynthetic microorganisms can be  
55 divided into three families: i) structural polysaccharides from cell walls, ii) storage

56 polysaccharides which are intracellular and iii) matricial polysaccharides released into the  
57 environment. Some of them are not completely released into the medium and remain linked to  
58 the cell. So, matricial polysaccharides were split into two categories by authors: i) those which  
59 are retained to the cell and called cell-bound polysaccharides or capsular polysaccharides and  
60 ii) those completely released into the environment called, extracellular polysaccharides,  
61 extracellular polymeric substance or exopolysaccharides (EPS). Today, there is always a thin  
62 line between these categories and the use of “EPS” is often clumsily used to qualify cell bound  
63 polysaccharides. The first publication focusing on EPS from microalgae was published in 1964  
64 by Tischer and Moore and revealed the composition of a polymer produced by *Palmella mucosa*  
65 [8]. Since the number of species of microalgae/cyanobacteria producing EPS described in  
66 literature has increased at a moderate level. Even if the effects of environment (culture  
67 conditions) on the EPS production by microalgae and cyanobacteria appear as species  
68 dependent, nutrient limitation and notably nitrogen starvation is the most described  
69 environmental constraint leading to EPS synthesis [7]. The main families characterized are  
70 Desmidiaceae, Chlamydomonadaceae, Chlorellaceae, Porphyridiaceae and Glaucosphaeraceae  
71 in microalgae, and Nostocaceae, Oscillatoriaceae and Microcoleaceae in cyanobacteria. Note  
72 also that some microalgae belonging to the Chromista kingdom and more especially those from  
73 the Bacillariophyceae class have also been described for the production of EPS. This rise is not  
74 really correlated with the large taxonomic diversity of microalgae despite the detection of *in*  
75 *vivo* and *in vitro* biological activities associated to the presence of these biopolymers such as  
76 immunomodulatory [9], anti-oxidative [10], anti-inflammatory [11], anticoagulant [12],  
77 antibacterial [13], anticancer [14], or anti-parasitic [15] properties. Some interesting rheological  
78 properties revealed also the potential of these EPS as gelling and thickening agents [7]. Based  
79 on these observations but also on the large diversity of unstudied microalgae, these  
80 microorganisms are very attractive regarding the production of new EPS.

81 The aim of this study was to increase the knowledge about the diversity of  
82 microalgae/cyanobacteria producing original EPS and to screen them for this phenotype. It has  
83 been done as part of the French Polysalgue project funded by the French National Research  
84 Agency (ANR) and aims to identify new strains of microalgae and cyanobacteria for the  
85 production of high value EPS. Previous screening studies had been done by De Philippis et al.  
86 [16, 17] but it was restricted to cyanobacteria, in 15 and 25 strains of the *Cyanothece* and *Nostoc*  
87 genera, respectively. In this work 166 original strains including 150 microalgae and 16  
88 cyanobacteria from diverse phylogenetic groups collected and distributed by the Roscoff  
89 Culture Collection (RCC), France (<http://roscoff-culture-collection.org/>) were screened for the  
90 production of EPS. Strains were selected based on their phylogenetic diversity, their promising  
91 phenotype and their belonging to species not previously described as EPS producers. Positive  
92 strains were able to grow in the under choosing conditions of culture and to produce a minimum  
93 of 0.05 g.L<sup>-1</sup> of extracellular carbohydrates after 30 days of culture. The results obtained were  
94 discussed and compared to the literature in order to highlight a possible relationship between  
95 phylogenetic microalgae groups and EPS production/composition.

## 96 **2. Material and methods**

### 97 **2.1. Screening procedure**

98 The screening was performed on 166 marine microalgae and cyanobacteria species from  
99 the RCC (**Table 1 and Supplementary data 1 Figure S1**). The f/2 medium was used for  
100 microalgae cultivation [18]. It was composed of NaNO<sub>3</sub> 0.075 g, NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O 0.0055 g,  
101 Na<sub>2</sub>EDTA 0.00416 g, FeCl<sub>3</sub>.6H<sub>2</sub>O 0.00315 g, CuSO<sub>4</sub>.5H<sub>2</sub>O 0.00001 g, ZnSO<sub>4</sub>.7H<sub>2</sub>O 0.000022  
102 g, CoCl<sub>2</sub>.6H<sub>2</sub>O 0.00001 g, MnCl<sub>2</sub>.4H<sub>2</sub>O 0.00018 g, Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O 0.000006 g,  
103 cyanocobalamin (B12) 0.0000005 g, thiamin (B1) 0.0001 g and biotin 0.0000005 g dissolved  
104 in 1 L of artificial sea water (ASW). ASW was composed of NaCl 28.13 g, KCl 0.77 g,  
105 CaCl<sub>2</sub>.2H<sub>2</sub>O 1.60 g, MgCl<sub>2</sub>.6H<sub>2</sub>O 4.80 g, NaHCO<sub>3</sub> 0.11 g, MgSO<sub>4</sub>.7H<sub>2</sub>O 3.50 g dissolved in 1

106 L of milliQ water. The pH was adjusted to 8 - 8.2 and the medium was sterilized by filtration  
107 (0.22  $\mu\text{m}$ ). The medium used for cyanobacteria was the PCR-S11 described by the RCC  
108 (<http://roscoff-culture-collection.org/medium-id/pcr-s11-red-sea>) and adapted from [19]. It was  
109 composed of one liter of Red Sea salt solution at 33.3  $\text{g}\cdot\text{L}^{-1}$  supplemented by 1 mL of HEPES-  
110 NaOH 1 M (pH 7.5) buffer, 1 mL of Na<sub>2</sub>-EDTA/FeCl<sub>3</sub> solution, 1 mL of Na-PO<sub>4</sub> 50 mM (pH  
111 7.5) buffer, 1 mL of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution (400 mM), 100  $\mu\text{L}$  of trace metals « Gaffron+Se » and  
112 100  $\mu\text{L}$  of vitamin B12 (10  $\text{mg}\cdot\text{mL}^{-1}$ ). The red Sea salt solution was sterilised at 121 °C during  
113 20 min whereas the other solutions were filter-sterilized (0.22  $\mu\text{m}$ ). The HEPES-NaOH 1 M (pH  
114 7.5) buffer was composed of 119.5 g of HEPES dissolved in 250 mL of milliQ water. The pH  
115 of the solution was adjusted to 7.5 before diluting with milliQ water to a final volume of 500  
116 mL. The Na<sub>2</sub>-EDTA/FeCl<sub>3</sub> complex solution was prepared dissolving FeCl<sub>3</sub>.6H<sub>2</sub>O (1.08 g) and  
117 Na<sub>2</sub>-EDTA (1.488 g) in 40 ml of HCl (0.1 M) and 40 ml of NaOH (0.1 M), respectively, before  
118 mixing and diluting with milliQ water to a final volume of 2000 mL. The « Gaffron+Se » trace  
119 metal solution used contained for one litre of milliQ water H<sub>3</sub>BO<sub>3</sub> (0.186 g), MnSO<sub>4</sub>.H<sub>2</sub>O (0.101  
120 g), (NH<sub>4</sub>)<sub>6</sub>MO<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O (0.00198 g), KBr (0.00516), KI (0.00714 g), ZnSO<sub>4</sub>.7H<sub>2</sub>O (0.00498  
121 g), (Cd(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O (0.01725 g), Co(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O (0.00925 g), CuSO<sub>4</sub>.5H<sub>2</sub>O (0.00876 g),  
122 NiCl<sub>2</sub>.6H<sub>2</sub>O (0.0071 g), Cr(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O (0.0024 g), VOSO<sub>4</sub>.5H<sub>2</sub>O (0,0015 g),  
123 KAl(SO<sub>4</sub>)<sub>2</sub>.12H<sub>2</sub>O (0.0284 g) and SeO<sub>2</sub> (0.0033 g). Finally, the Na-PO<sub>4</sub> 50 mM (pH 7.5) buffer  
124 was composed of 6 g of NaH<sub>2</sub>PO<sub>4</sub> dissolved in 1 litre of milliQ water mixed with 3.55 g of  
125 Na<sub>2</sub>HPO<sub>4</sub> dissolved in 500 mL of milliQ water. The pH of the buffer was then adjusted to 7.5.  
126 Strains were cultivated photoautotrophically during 30 days in 50 mL flasks containing 20 mL  
127 of medium inoculated with 4 mL of microorganism culture. Cultures were performed at 20 °C  
128 under stirring (120 rpm). The values of irradiance were 150 and 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  in parallel  
129 with a light:dark regime (16h: 8h). These irradiance values were chosen in order to be suitable  
130 for strains growing at low (150  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ ) and high (300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ ) light intensities in

131 accordance with literature [3, 7]. This also allowed studying the impact of the light intensity on  
132 the production of EPS for further experiments. Samples of 1 mL were collected throughout the  
133 process to follow cell growth, nitrate consumption and extracellular sugars production.

## 134 **2.2. Growth, nitrate consumption and EPS production measurements**

135 Growth of microalgae/cyanobacteria was followed by spectrophotometry ( $A_{750}$ ). This  
136 wavelength was chosen to avoid interferences with the pigments. Nitrate and ammonium  
137 concentrations were measured on supernatant of culture samples treated by centrifugation (10  
138 000 g for 10 min at 20 °C) according to the method of Caswe et al. [20] modified by A.P.H.A  
139 [21], and Patton and Crouch [22], respectively. Total sugars contents in the same supernatants  
140 desalted using a 10 kDa membrane were determined according to the phenol sulphuric assay  
141 [23] and expressed as D-glucose equivalent ( $\text{g.L}^{-1}$ ).

## 142 **2.3. Exopolysaccharide production**

143 Strains identified as potential EPS producers were transferred to a modified f/2 medium  
144 supplemented in nitrogen and phosphorous sources (1 g of  $\text{NaNO}_3$  and 0.2 g of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ )  
145 and called enriched f/2 medium. These concentrations were determined based on a  
146 stoichiometric approach [24-25]. Strains were maintained photoautotrophically in 500 mL  
147 flasks containing 200 mL of enriched f/2 medium inoculated with 20 mL of microalgae culture  
148 in exponential phase of growth. Culture conditions were similar to those of screening. Strains  
149 were let growing in these conditions during 60 days in order to maximize the biomass  
150 production, including EPS.

## 151 **2.4. Exopolysaccharide extraction**

152 Cultures were centrifuged at 10 000 g for 15 min at 20 °C. EPS in supernatants were  
153 desalted by cycles of concentrations-dilutions (milliQ water) using an Amicon centrifugal filter  
154 equipped with a 10 kDa NMWCO membrane. EPS were considered as purified when the  
155 conductivity of the filtrates was near to that of milliQ water (around  $2 \mu\text{S.cm}^{-1}$ ). Finally, EPS



156 solutions were freeze-dried during 48 h and samples were stored at room temperature until  
157 further analyses.

## 158 **2.5. Monosaccharide compositions**

159 Monosaccharides compositions of EPS were both determined by High-Pressure Anion  
160 Exchange Chromatography equipped with Pulsed Amperometric Detection (HPAEC-PAD) and  
161 Gas Chromatography coupled to Mass Spectrophotometer with Electron Ionization (GC/MS-  
162 EI) to ensure data complementary. For both methods results were expressed in molar ratio  
163 percentages.

164 For HPAEC-PAD analyses, 10 mg of EPS were dissolved in 1 mL of 2 M trifluoroacetic  
165 acid (TFA) solution in a glass tube and heated at 120 °C for 90 min. The samples were stirred  
166 3 times during the hydrolysis. After hydrolysis, pH was adjusted to 7 by addition of ammonium  
167 hydroxide (35 % w/v) and the solutions were centrifuged at 14 000 g for 15 min at room  
168 temperature. Supernatants were filtered through 0.2 µm membrane filter and analyzed by  
169 HPAEC with an ICS 3000 system (Dionex Corporation, Sunnyvale (CA), USA) equipped with  
170 pulsed amperometric detection (PAD) and AS 50 autosampler. Twenty-five µL of samples were  
171 injected in the system and eluted into a pre-column CarboPac PA1-column (4×50 mm) and an  
172 analytical CarboPac PA1-column (4×250 mm) equilibrated 15 min with 18 mM NaOH.  
173 Samples were eluted isocratically with 18 mM NaOH for 25 min, followed by a linear gradient  
174 between 0 to 0.5 M sodium acetate in 200 mM NaOH for 20 min to elute acidic  
175 monosaccharides. Run was followed by 15 min washing with 200 mM NaOH. The eluent flow  
176 rate was kept constant at 1 mL.min<sup>-1</sup>. Columns were thermostated at 25 °C. Data were collected  
177 and analyzed with Dionex Chromeleon 6.80 software (Sunnyvale, USA). L-Rha, D-Rib, L-Fuc,  
178 L-Ara, D-Xyl, D-Man, D-Gal, D-Glc, D- GlcA, and D-GalA were used as standards.

179 For GC/MS analyses, 15 mg of EPS were dissolved in 1.5 mL of TFA in a glass tube  
180 followed by heating at 120 °C for 90 min. The samples were mixed each 30 min. The

181 preparations were then evaporated under nitrogen stream. The dried residues were dissolved in  
182 BSTFA: TMCS (99: 1) following the procedure described by Pierre et al. [26-27], then  
183 evaporated again under nitrogen stream. Dichloromethane was finally added to the derivatized  
184 products prior to injection onto GC/MS-EI apparatus. Standards (L-Rha, D-Rib, L-Fuc, L-Ara,  
185 D-Xyl, D-Man, D-Gal, D-Glc, D-GlcA, D-GalA, D-GlcN, D-GalN) were prepared following the  
186 same procedure. Analyses were carried out by GC/MS-EI using an Agilent 6890 Series GC  
187 System coupled to an Agilent 5973 Network Mass Selective detector. Derivatives were  
188 analyzed using an OPTIMA-1MS (30 m, 0.32 mm, 0.25 mm) column, under helium flow of 2.3  
189 mL.min<sup>-1</sup>. The helium pressure was set to 8.8 psi and the split ratio to 50:1. The temperature  
190 was programmed to 100 °C for 3 min then raised to 200 °C at 8 °C.min<sup>-1</sup> keeping for 1 min then  
191 continuing with a final temperature increment to 215 °C at 5 °C.min<sup>-1</sup>. The ionization was  
192 carried out by Electronic Impact (EI, 70 eV). The trap temperature was fixed to 150 °C and the  
193 target ion was from 40 to 800 m/z. Data processing was done with LC/MSD Trap Version 5.3 and  
194 MestreNova Version 7.1 Softwares (Agilent).

## 195 **2.6. Relationship between phylogeny and exopolysaccharide composition**

### 196 **2.6.1. Statistics analysis**

197 Differences in levels of each monosaccharide were tested between 5 of the 6  
198 microalgae phyla. Miozoa were not included in the analysis, since only two monosaccharide  
199 compositions were available. For each monosaccharide, Kruskal-Wallis tests were performed.  
200 When a significant difference was found ( $p < 0.05$ ), this test was followed by multiple pairwise  
201 comparisons between phyla (Mann-Whitney tests), with significance levels adjusted according  
202 to the Benjamini and Hochberg method [28].

### 203 **2.6.2. Phylogenetic tree**

204 Monosaccharide compositions of 81 EPS from microalgae (including those from the  
205 strains CCAP 904/1, RCC4633, RCC3686, RCC6688, RCC3514, RCC1544, RCC1545,

206 RCC4037, RCC4120, RCC3438, RCC3688, RCC3685, RCC3707, RCC2380, RCC1564,  
207 RCC133, RCC4435, RCC5, RCC2684 and RCC4628), belonging to 6 major phyla and 15  
208 different classes, were compiled in a phylogenetic tree. The classification used was that of  
209 Algaebase database (<http://www.algaebase.org>). Algaebase is an on-line database providing  
210 free access to authoritative taxonomic, distributional and nomenclatural information [29]. The  
211 nucleotide sequences coding for 18S ribosomal RNA (18S rRNA) were searched on the  
212 National Center for Biotechnology Information (NCBI) database for all these strains. For 41  
213 species, 1 to 3 sequences were found on this database. For the others, sequences obtained from  
214 species belonging to the same genus were used. Other sequences have also been added to  
215 construct a phylogenetic tree consistent with recently published phylogenetic trees [30-32]. 214  
216 sequences were obtained and aligned using the MUSCLE algorithm [33] available in the  
217 MEGA 7 software [34]. Then the tree was constructed using the Neighbor-Joining method with  
218 the MEGA 7 software over a total of 1248 positions, based on the p-distance method with 2000  
219 bootstraps. All positions containing gaps and missing data were eliminated. The monosaccharide  
220 compositions (in % of the total composition or presence/absence) of 81 strains were added to  
221 this phylogenetic tree using the iTOL tool [35]. All classes were supported by bootstrap values  
222 higher than 0.92, except for Zygnematophyceae (0.55), Chlorophyceae (0.54) and  
223 Trebouxiophyceae (0.35).

### 224 **3. Results and Discussion**

#### 225 **3.1. Screening of strains with EPS+ phenotype**

226 Hundred and sixty-six (150 microalgae and 16 cyanobacteria) strains were selected from  
227 the 5852 strains of the RCC based on the following criteria: i) cover a maximum of phylogenetic  
228 diversity, ii) target classes and orders not described for the production of EPS, iii) select  
229 different species to study the diversity of compositions within the same genus and iv) choose  
230 strains with a “promising phenotype” as both biofilm and gel formations or slimy appearance

231 during the cultures. Short morphologic descriptions of strains for which genus and species names are  
232 not known are available on the Roscoff Culture Collection (<http://roscoff-culture-collection.org/>). To  
233 identify positive strains two conditions were required: growing under the chosen conditions and  
234 producing a minimum of  $0.05 \text{ g.L}^{-1}$  of true EPS at D30 (30 days). These criteria were evaluated  
235 thanks to the results of  $A_{750}$ , nitrate (or ammonium) and total carbohydrates assays (**Table 1;**  
236 **Supplementary data Figure S1**). In support of these results, the 166 strains were classified  
237 into three groups.

**Table 1 part 1:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>‡</sup>	Dubois assay [22]	Groups
			Chroococcales	Microcystaceae	<i>Microcystis</i>	sp.	26	+	0.00	EPS-
			Nostocales	Aphanizomenonaceae	<i>Nodularia</i>	<i>spumigena</i>	4299	+	0.00	EPS-
			Pleurocapsales	Xenococcaceae	<i>Xenococcus</i>	sp.	2703	+	0.00	EPS-
			Spirulinales	Spirulinaceae	<i>Spirulina</i>	sp.	1786	+	0.00	EPS-
				Acaryochloridaceae	<i>Acaryochloris</i>	<i>marina</i>	1983	+	0.00	EPS-
						<i>tomasi</i>	1774	+	0.00	EPS-
				Leptolyngbyaceae	<i>Leptolyngbya</i>	sp.	232	+	0.00	EPS-
				Prochloraceae	<i>Prochlorococcus</i>	sp.	269	+	0.00	EPS-
Bacteria	Cyanobacteria	Cyanophyceae			<i>Cyanobium</i>	sp.	2436	+	0.00	EPS-
			Synechococcales				2380	+	<b>0.12*</b>	<b>EPS+</b>
							1084	+	0.00	EPS-
				Synechococcaceae			2035	+	0.00	EPS-
					<i>Synechococcus</i>	sp.	2368	+	0.00	EPS-
							2381	+	0.00	EPS-
							2383	+	0.00	EPS-
							4555	+	0.00	EPS-

The classification was done according to the AlgaeBase database [29]. ☐ no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer,

NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and \*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>‡</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 2:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups	
Chromista	Ochrophyta	Bacillariophyceae	Bacillariales	Bacillariaceae	<i>Cylindrotheca</i>	sp.	2608	-	-	NGS	
						<i>Nitzschia</i>	sp.	2638	-	-	NGS
			Fragilariales					2598	+	0.00	EPS-
			Naviculales	Naviculaceae	<i>Navicula</i>	sp.	3092	-	-	NGS	
		Chlorarachniophyceae	Chlorarachniales	Chlorarachniaceae	<i>Bigelowiella</i>	<i>natans</i>	2352	+	<b>0.05*</b>	<b>EPS+</b>	
		Coccinodiscophyceae	Rhizosoleniales	Rhizosoleniaceae	<i>Guinardia</i>	<i>flaccida</i>	3093	-	-	NGS	
			Chromulinales	Chromulinaceae	<i>Ochromonas</i>	sp.	2350	-	-	NGS	
				Chrysamoebaceae	<i>Chrysamoeba</i>	sp.	377	-	-	NGS	
					<i>Chroomonas</i>	sp.	1504	-	-	NGS	
			Cryptomonadales	Hemiselmidaceae		sp.	3436	+	0.02	EPS-	
			Cryptophyceae			<i>Hemiselmis</i>	sp.	2614	-	-	NGS
				Geminigeraceae	<i>Proteomonas</i>	sp.	3072	+	0.02	EPS-	
			Pyrenomonadales			<i>sulcata</i>	3649	-	-	NGS	
				Pyrenomonadaceae	<i>Rhinomonas</i>	sp.	821	+	0.00	EPS-	
						sp.	1978	+	0.02	EPS-	
			Dictyochophyceae	Florenciellales		<i>Pseudochattonella</i>	<i>verruculosa</i>	1082	-	-	NGS
				Rhizochromulinales	Rhizochromulinaceae	<i>Rhizochromulina</i>	sp.	4438	-	-	NGS

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and \*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 3:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>‡</sup>	Dubois assay [22]	Groups				
Chromista	Ochrophyta	Mediophyceae	Chaetocerotales	Chaetocerotaceae	<i>Chaetoceros</i>	sp.	2564	-	-	NGS				
						<i>Papiliocellulus</i>	sp.	2624	+	0.00	EPS-			
			Cymatosirales	Cymatosiraceae	<i>Minutocellulus</i>	sp.	1863	-	-	NGS				
		Mediophyceae	Eupodiscales	Eupodiscaeae		<i>Odontella</i>	sp.	2558	-	-	NGS			
							Lithodesmiales	Lithodesmiaceae	<i>Ditylum</i>	<i>brightwellii</i>	775	-	-	NGS
							Thalassiosirales	Thalassiosiraceae	<i>Skeletonema</i>	sp.	2932	-	-	NGS
			<i>Minidiscus</i>	<i>trioculatus</i>	4657	-				-	NGS			
			<i>Thalassiosira</i>	sp.	1714	-				-	NGS			
							<i>weissflogii</i>	76	-	-	NGS			
			Pelagophyceae	Pelagomonadales	Pelagomonadaceae		<i>Aureococcus</i>	<i>anophagefferens</i>	96	-	-	NGS		
								sp.	4094	-	-	NGS		
								<i>Pelagococcus</i>	sp.	3069	+	<b>0.10**</b>	<b>EPS+</b>	
		Sarcinochrysidales		Sarcinochrysidaceae		<i>Andersenina</i>	<i>nodulosa</i>	4631	+	0.01	EPS-			
							<i>Aureoumbra</i>	<i>lagunensis</i>	97	-	-	NGS		
											2696	+	0.01	EPS-
Phaeothamniophyceae	Aurearenales	Aurearenaceae		<i>Aurearena</i>	<i>cruciata</i>	4621	+	0.01	EPS-					
Pinguiphyceae	Pinguiochrysidales	Pinguiochrysidaceae		<i>Glossomastix</i>	sp.	1196	+	0.01	EPS-					

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and \*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>‡</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 4:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups
						sp.	3685	+	<b>0.06*</b>	EPS+
		Pinguiphyceae	Pinguiochrysidales	Pinguiochrysidaceae	<i>Glossomastix</i>	sp.	3688	+	<b>0.10**</b>	EPS+
						sp.	3707	+	<b>0.11*</b>	EPS+
	Ochrophyta				<i>Phaeomonas</i>	sp.	4470	+	<b>0.07**</b>	EPS+
		Raphidophyceae	Chattonellales	Fibrocapsaceae	<i>Fibrocapsa</i>	<i>japonica</i>	1501	-	-	NGS
					<i>Heterosigma</i>	<i>akashino</i>	1502	+	0.01	EPS-
		Synchromophyceae	Synchromales	Synchromaceae	<i>Guanochochroma</i>	<i>wildpretii</i>	3390	-	-	NGS
							CC 19	+	<b>0.13*</b>	EPS+
Chromista					<i>Calcidiscus</i>	<i>leptoporus</i>	1170	-	-	NGS
				Calcidiscaceae	<i>Oolithotus</i>	<i>fragilis</i>	2321	-	-	NGS
						sp.	1180	+	<b>0.77**</b>	EPS+
				Calyptosphaeraceae	<i>Calyptosphaera</i>	<i>sphaeroidea</i>	1178	-	-	NGS
	Haptophyta	Coccolithophyceae	Coccolithales	Coccolithaceae	<i>Coccolithus</i>	<i>braarudii</i>	3777	-	-	NGS
					<i>Hymenomonas</i>	<i>coronata</i>	1337	+	<b>0.05*</b>	EPS+
						<i>verrucosa</i>	3650	+	<b>0.05*</b>	EPS+
				Hymenomonadaceae	<i>Ochrosphaera</i>		3635	+	<b>0.05*</b>	EPS+
						sp.	2976	-	-	NGS

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and\*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.



**Table 1 part 5:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups
						sp.	4633	+	<b>0.22*</b>	<b>EPS+</b>
			Isochrysidales	Isochrysidaceae	<i>Chrysothila</i>	sp.	CCAP 904/1	+	<b>0.16*</b>	<b>EPS+</b>
						<i>roscoffensis</i>	1395	+	0.03	EPS-
					<i>Isochrysis</i>	<i>braarudii</i>	3686	+	<b>0.06*</b>	<b>EPS+</b>
				Isochrysidaceae	<i>Ruttnera</i>	<i>lamellosa</i>	4061	+	<b>0.05*</b>	<b>EPS+</b>
			Isochrysidales		<i>Tisochrysis</i>	<i>lutea</i>	3699	+	0.02	EPS-
							1216	+	<b>0.07**</b>	<b>EPS+</b>
				Noelaerhabdaceae	<i>Emiliana</i>	<i>huxleyi</i>	1217	+	0.02	EPS-
					<i>Gephyrocapsa</i>	<i>oceanica</i>	1314	-	-	NGS
Chromista	Haptophyta	Coccolithophyceae					739	-	-	NGS
					<i>Phaeocystis</i>	<i>globosa</i>	1719	-	-	NGS
							851	-	-	NGS
							908	-	-	NGS
			Phaeocystales	Phaeocystaceae			925	-	-	NGS
					<i>Phaeocystis</i>	sp.	940	-	-	NGS
							2055	-	-	NGS
							3539	-	-	NGS
			Prymnesiales	Prymnesiaceae	<i>Prymnesium</i>	<i>parvum</i>	3426	+	<b>0.12*</b>	<b>EPS+</b>

The classification was done according to the AlgaeBase database [29]. ☐ no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and\*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 6:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups
				Prymnesiaceae	<i>Imantonia</i>	<i>rotunda</i>	1342	+	0.04	EPS-
			Prymnesiales		<i>Haptolina</i>	<i>hirta</i>	3421	-	-	NGS
		Coccolithophyceae		Chrysochromulinaceae	<i>Chrysochromulina</i>	<i>camella</i>	1186	-	-	NGS
			Zygodiscales	Pontosphaeraceae	<i>Scyphosphaera</i>	<i>apsteinii</i>	1456	-	-	NGS
							3704	+	<b>0.05*</b>	<b>EPS+</b>
						sp.	3514	+	<b>0.29**</b>	<b>EPS+</b>
					<i>Diacronema</i>	<i>lutheri</i>	1537	+	0.01	EPS-
						<i>viridis</i>	3459	+	0.02	EPS-
Chromista	Haptophyta						1536	+	<b>0.06*</b>	<b>EPS+</b>
					<i>Exanthemachrysis</i>	sp.	1544	+	<b>0.10*</b>	<b>EPS+</b>
						<i>enorae</i>	1525	+	<b>0.05*</b>	<b>EPS+</b>
		Pavlovophyceae	Pavloales	Pavlovaceae		<i>gyrans</i>	1545	+	<b>0.05*</b>	<b>EPS+</b>
							4037	+	<b>0.09*</b>	<b>EPS+</b>
					<i>Pavlova</i>		4120	+	<b>0.08*</b>	<b>EPS+</b>
						sp.	4070	+	<b>0.07*</b>	<b>EPS+</b>
							3438	+	<b>0.39*</b>	<b>EPS+</b>
					<i>Rebecca</i>	sp.	1528	+	<b>0.05**</b>	<b>EPS+</b>

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and \*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 7:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups
	Haptophyta						6688	+	<b>0.10*</b>	<b>EPS+</b>
			Gonyaulacales	Ostreopsidaceae	<i>Alexandrium</i>	<i>minutum</i>	1490	+	0.01	EPS-
				Brachidiniaceae	<i>Karenia</i>	<i>brevis</i>	4635	-	-	NGS
						<i>mikimotoi</i>	1513	-	-	NGS
			Gymnodiniales		<i>Amphidinium</i>	<i>carterae</i>	1522	+	<b>0.05**</b>	<b>EPS+</b>
				Gymnodiniaceae	<i>Cochlodinium</i>	<i>polykrikoides</i>	4634	-	-	NGS
					<i>Lepidodinium</i>	<i>chlorophorum</i>	1489	-	-	NGS
			Peridinales	Heterocapsaceae	<i>Heterocapsa</i>	sp.	3466	+	0.01	EPS-
Chromista	Miozoa	Dinophyceae					2616	-	-	NGS
			Prorocentrales	Prorocentraceae	<i>Prorocentrum</i>	<i>micans</i>	1517	-	-	NGS
					<i>Biecheleriopsis</i>	sp.	3656	-	-	NGS
			Suessiales	Suessiaceae	<i>Pelagodinium</i>	sp.	3003	-	-	NGS
					<i>Symbiodinium</i>	<i>kawagutii</i>	4019	+	0.02	EPS-
						<i>voratum</i>	1521	+	0.03	EPS-
			Thoracosphaerales	Thoracosphaeraceae	<i>Thoracosphaera</i>	<i>heimii</i>	1512	+	0.01	EPS-
					<i>Scrippsiella</i>	<i>trochoidea</i>	1515	+	0.01	EPS-

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and \*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 8:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups
						<i>chui</i>	128	+	<b>0.05*</b>	EPS+
						<i>globosa</i>	1564	+	<b>0.05*</b>	EPS+
		Chlorodendrophyceae	Chlorodendrales	Chlorodendraceae	<i>Tetraselmis</i>	<i>rubens</i>	133	+	<b>0.05*</b>	EPS+
						<i>striata</i>	130	+	0.02	EPS-
						sp.	4435	+	<b>0.21**</b>	EPS+
							119	+	0.03	EPS-
Plantae	Chlorophyta			Chlamydomonadaceae	<i>Chlamydomonas</i>	sp.	3443	+	0.01	EPS-
		Chlorophyceae	Chlamydomonadales	Dunaliellaceae	<i>Dunaliella</i>	sp.	5	+	<b>0.06**</b>	EPS+
						<i>tertiolecta</i>	6	+	0.02	EPS-
				Bathycoccaceae	<i>Bathycoccus</i>	<i>prasinos</i>	4222	-	-	NGS
		Mamiellophyceae	Mamiellales	Mamiellaceae	<i>Micromonas</i>	<i>commoda</i>	827	+	0.01	EPS-
					<i>Ostreococcus</i>	<i>tauri</i>	1108	+	0.01	EPS-
		Nephrophyceae	Nephroselmidales	Nephroselmidaceae	<i>Nephroselmis</i>	sp.	1805	+	0.03	EPS-

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and\*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 9:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups
Chlorophyta	Palmophyllophyceae	Prasinococcales	Prasinococcaceae	<i>Prasinococcus</i>	sp.	2684	+	<b>0.07*</b>	EPS+	
				<i>Prasinoderma</i>	sp.	2686	+	0.02	EPS-	
	Picocystophyceae	Picocystales	Picocystaceae	<i>Picocystis</i>	<i>salinarum</i>	3402	+	0.02	EPS-	
	Pyramimonadophyceae	Pseudoscourfieldiales	Pycnococcaceae	<i>Pycnococcus</i>	<i>provasolii</i>	2336	+	0.01	EPS-	
				<i>Pyramimonas</i>	sp.	4212	-	-	NGS	
		Pyramimonadales	Pyramimonadaceae	<i>Chlorella</i>	sp.	2340	+	<b>0.05*</b>	EPS+	
	Plantae	Trebouxiophyceae	Chlorellales	Chlorellaceae	<i>Nannochloris</i>	sp.	3644	+	0.00	EPS-
					<i>Picochlorum</i>	sp.	3065	+	0.00	EPS-
					<i>Coccomyxa</i>	sp.	903	+	<b>0.05*</b>	EPS+
							3440	-	-	NGS
Rhodophyta	Porphyridiophyceae	Porphyridiales	Porphyridiaceae	<i>Porphyridium</i>	sp.	4628	+	<b>0.23*</b>	EPS+	
				<i>Rhodella</i>	<i>maculata</i>	655	+	<b>0.06*</b>	EPS+	
	Stylonematophyceae	Stylonematales	Stylonemataceae	<i>Chroodactylon</i>	<i>ramnosum</i>	654	+	0.01	EPS-	
				<i>Stylonema</i>	sp.	2964	+	0.00	EPS-	

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, NGS: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and\*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.

**Table 1 part 10:** Growth ( $A_{750}$ ), production of extracellular carbohydrates and phenotype of 166 microalgae and cyanobacteria from the RCC.

Kingdoms	Phyla	Classes	Orders	Families	Genera	Species	RCC numbers	Growth ( $A_{750}$ ) <sup>±</sup>	Dubois assay [22]	Groups
							3452	+	0.06*	EPS+
							A13 880	+	0.08*	EPS+
							CC 21	+	0.05*	EPS+
							CC 76	+	0.05*	EPS+
							3641	+	0.01	EPS-
							4078	+	0.01	EPS-
							18/1	+	0.00	EPS-
							AST 713	+	0.01	EPS-
							CC 14	+	0.01	EPS-
							CC 20	+	0.02	EPS-
							3448	-	-	NGS
							3473	-	-	NGS
							AST 714	-	-	NGS
							JAP PR7	-	-	NGS
							JAP PR8	-	-	NGS
							KO 334	-	-	NGS
							OSH 51	-	-	NGS

The classification was done according to the AlgaeBase database [29]. ☐: no classification available. +: positive result, -: negative result, **EPS+**: positive strain, EPS producer, **EPS-**: negative strain, no EPS producer, **NGS**: Not Growing-Strain, \* value obtained at 150 and  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  and\*\* value obtained at 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , <sup>±</sup>growth expressed as significant increase of  $A_{750}$  in time.

The first group (EPS+) was composed of strains (45/166) for which the total carbohydrates assays [23] gave a positive result for at least one of the two light intensities. The Table 1 gives the higher value of EPS production (obtained with an irradiance of 150 or 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ ) for each strain. Some strains EPS+ produced EPS at the two irradiances (strains A13 880, CC19, CCAP904/1, RCC1180, RCC1544, RCC1545, RCC2684, RCC3426, RCC3438, RCC3514, RCC3685, RCC3688, RCC3707, RCC4037, RCC4120, RCC4435, RCC4470, RCC4628, RCC4633 and RCC6688) whereas some other strains were detected as EPS producers at only 150  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  (CC21, CC76, RCC128, RCC133, RCC655, RCC903, RCC1337, RCC1525, RCC1536, RCC1564, RCC2340, RCC2352, RCC2380, RCC3452, RCC3635, RCC3650, RCC3686, RCC3704 and RCC4070) or 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  (RCC5, RCC1216, RCC1522, RCC1528 and RCC3069) irradiances. For the major part of these strains, the production of exocellular carbohydrates was correlated with a nitrogen starvation into the culture medium, as described in the literature [7]. This group included microalgae and cyanobacteria, together representing 7 phyla, 13 classes, 15 orders, 18 families and 24 genera. It was essentially composed of eukaryotes belonging to Chromista (30 species) or Plantae (10 species) kingdoms. Surprisingly, the *Synechococcus* sp., previously known for EPS production, was the sole member of prokaryotic domain whereas a large number of Cyanobacteria has been described in previous studies for the production of EPS [36-37]. This observation could be explained by the very low number of screened cyanobacteria (only 16) but also by their ability to produce cell bound polysaccharides which are not measured in the extracellular medium. Four microalgae could not be phylogenetically classified due to a lack of information available in the RCC. The strains EPS+ from the kingdom Chromista belong mainly to the Haptophyta and Ochrophyta phyla. Haptophyta are essentially represented by the Coccolithophyceae and Pavlovophyceae classes. The family Isochrysidaceae was the most represented in Coccolithophyceae with 45 % of EPS+ strains. The family Pavlovaceae was the only

representative of Pavlovophyceae. Among the Ochrophyta, the Pinguiochrysidaceae family included four strains out of five EPS+, ones belonging to the genera *Phaeomonas* and *Glossomastix*. The strains found EPS+ in the kingdom Plantae belong to the Chlorophyta and Rhodophyta phyla. In Chlorophyta, the family Chlorodendraceae was detached from others as it accounted for 40 % of EPS+ strains. All these strains belong to the sole *Tetraselmis* genus. Regarding the Rhodophyta, two EPS+ strains belonging to the genera *Rhodella* and *Porphyridium*, which are well known in literature as EPS producers were detected [7].

The second group included strains not detected as EPS producers (EPS-) in the tested culture conditions. It was composed of 46 strains of microalgae and 15 strains of cyanobacteria. These strains are spread among 7 phyla, 20 classes, 29 orders, 34 families and 42 genera. Cell growth has been observed for all of them but not EPS production. For many microalgae strains, the release of EPS in the environment has been described to occur in response to a “stress”, relating to a nutrient deficiency (nitrogen or phosphorous sources for instance), or the quality and the intensity of light or the salinity of the medium [38-44]. Nevertheless, the low amounts of nitrogen and phosphorous in the f/2 nutrient associated with growth cells observed by A<sub>750</sub> measurements and the depletion of nitrogen (due to nitrate consumption) showed a nutrient deficiency, without production of EPS. Some strains of the group EPS- were able to grow to 300  $\mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , suggesting that a high light intensity was not sufficient to induce an EPS production. Finally, other screening conditions may have prevented the EPS production, like inadequate pH, temperature or agitation for example. Indeed, in nature benthic microalgae are less tolerant to shear stress than pelagic ones [45]. Stirring at 120 rpm during the screening could affect the production of EPS for some strains. Another possible explanation would be the production of BPS, not measured by the methodology used during this work. Note also that despite their “promising phenotype” leading to their selection for this screening, these strains or a part of these strains could simply not be EPS producers.



The third group corresponded to the strains for which no growth was observed during the screening, called “Not Growth-Strain” (NGS). It was composed of 60 strains distributed into 5 phyla, 13 classes, 28 orders, 35 families and 42 genera. The lack of growth could come from the composition of the culture medium due to the possible lack of some essential elements. For example, the silica is mandatory for the development of the Bacillariophyta (diatoms belonging to the Ochrophyta) [46]. This hypothesis was supported by the fact that most of diatoms fall into this group (except two, which were found EPS-). The salinity of the medium (28 g.L<sup>-1</sup>) can also be questioned.

Some strains belonging to families present in EPS- and NGS groups, including microalgae and cyanobacteria were also present in the EPS+ group such as Synechococcaceae (Cyanobacteria), Chlorodendraceae (Chlorophyta), Isochrysidaceae and Pavlovaceae (Haptophyta). However most of the microalgae and cyanobacteria present in these two groups belong to classes, and more specifically to genera that do not appear in the group of EPS+ strains. **Table 2** presents an exhaustive list of strains identified in literature as EPS producers. Comparing the two data sets, most of them (20/25 total) belong to genera which were unknown for the production of soluble EPS. Additional analyzes should be needed to confirm the nature of extracellular sugars produced by EPS+ strains as EPS.

### **3.2. Exopolysaccharide production and analysis**

Cultures of EPS+ strains were carried out for 60 days in order to produce EPS which were subsequently extracted. Then analyses by HPAEC-PAD and GC/MS-EI were performed to validate the positive results from the colorimetric assays. Analyses concerned 5 of the 7 phyla and 7 of the 15 orders of the EPS+ strains (45 % of the total strains). Some analyses were also performed on several EPS+ strains from the same family, and sometimes on different species belonging to the same genus in order to study the variability of the compositions. **Table 3** shows the results obtained using each method. Despite few differences that can be observed between

the two analytical methods, the results of the compositions were found to be similar, with the same main monosaccharides (Pearson:  $R^2 = 0.963$  ;  $p < 0.001$ ). Among the 11 genera whose composition was analysed, 3 have been already described in literature, *i.e.* *Synechococcus* (RCC2380) [36], *Dunaliella* (RCC5) [43] and *Porphyridium* (RCC4628) [15, 47-49]. In this study the EPS from *Dunaliella* sp. (RCC5) was mainly composed of Gal, Rha, Xyl, Man and GalA (28, 21, 17, 13 and 11 %, respectively) with some traces of other monosaccharides (Glc, Ara and GlcA). It presents some similarity with that previously described by Mishra and Jha [43] from *D. salina* which was composed of Xyl, Gal and Glc.

**Table 2, part 1:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera (phyla)	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
<i>Achnanthes</i> (Oc)	<i>longipes</i>	+	+++	+	+	+	-	+	++	+	-	nd	Nd		[58]
	<i>coffeaeformis</i>	+	++	+	+	nd	nd	+	+++	+	nd	nd	Nd		
<i>Amphora</i> (Oc)	<i>holsatica</i>	+	nd	+	+	nd	nd	nd	+	+	nd	nd	Nd		[64]
	<i>rostrata</i>	+	++	+	+	nd	nd	+	+++	+	nd	+	+	pyruvate	[57]
<i>Ankistrodesmus</i> (Chl)	<i>densus</i>	+	++	+	+	nd	nd	+	+++	+	-	-	Nd	linkages	[66]
<i>Aulacoseira</i> (Oc)	<i>granulata</i>	+++	+	nd	+	+	-	+	+	++	nd	nd	Nd	GlcNAc	[67]
<i>Botryococcus</i> (Chl)	<i>braunii</i>	+	+++	+	tr	tr	-	tr	++	+	-	nd	+		[68]
<i>Bracteacoccus</i> (Chl)	sp.	++	++	+	+++	nd	nd	+	+	+	-	+	Nd		[56]
	<i>affinis</i>	+	+	+	-	nd	nd	-	+	-	nd	+	Nd		[69]
<i>Chaetoceros</i> (Oc)	<i>curvisetus</i>	+	++	-	-	nd	nd	-	+++	-	-	+	Nd	linkages	[70]
	<i>decipiens</i>	+++	++	-	+	nd	nd	+	+++	+	-	nd	Nd	linkages	[71]
	<i>agustae</i>	-	+	+	+++	++	-	+	-	+	-	nd	+	linkages	[72]
<i>Chlamydomonas</i> (Chl)	<i>corrosa</i>	-	+++	++	+	-	+	-	-	-	-	nd	+	linkages	[72]
	<i>humicola</i>	+	+	++	++	+	-	-	-	+++	-	nd	+		[73]

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Cha: Charophyta, Chl: Chlorophyta, Mi: Miozoa, Oc: Ochrophyta, Rh: Rhodophyta. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: N-acetyl-glucosamine, GalNAc: N-acetyl-galactosamine.

**Table 2, part 2:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera (phyla)	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
Chlamydomonas (Chl)	<i>peterfii</i>	-	+++	+	+	-	++	-	-	-	-	nd	+		[73]
	<i>reinhardtii</i>	+	+	+	+	-	+	-	-	+	+	nd	-	pyruvate	[74]
	<i>mexicana</i>	+	+	+	+++	nd	nd	+	++	+	+	nd	Nd		[75]
Chlamydomonas (Chl)	<i>sajao</i>	+	+++	++	+	nd	nd	+	-	+	-	nd	Nd		[73]
	<i>sajao</i>	-	+++	+	+	-	++	-	-	-	-	nd	+		[73]
	<i>stigmatophora</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd		[76]
	sp.	-	-	-	+	-	-	-	+	+	-	nd	Nd		[77]
	<i>autotrophica</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd		[48]
	<i>mirabilis</i> (678F)	+	+++	++	+	nd	nd	+	+	+	-	+	Nd		
	<i>mirabilis</i> (7410G)	+	+++	+	+	nd	nd	+	++	+	-	tr	Nd		[56]
Chlorella (Chl)	<i>ellipsoidea</i>	+	+++	+	+	nd	nd	++	+	+	+	+	Nd		
	<i>pyrenoidosa</i>	+	+	+	+	-	-	+	+	+	-	nd	Nd		[78]
	<i>vulgaris</i>	+	-	-	+	+	-	-	-	+	-	nd	Nd		
	<i>ellipsoidea</i>	-	-	+	+	+	-	-	+	-	-	nd	Nd		[77]
	sp.	+	-	+	+	+	-	-	-	-	-	nd	Nd		
Closterium (Cha)	sp.	+	+	+	+	+++	-	+	++	+	-	nd	Nd	linkages	[54]
Cochlodinium (Mi)	<i>polykrikoides</i>	-	+	-	+	+	+	+	-	-	-	+	-		[79]

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Cha: Charophyta, Chl: Chlorophyta, Mi: Miozoa, Oc: Ochrophyta, Rh: Rhodophyta. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: *N*-acetyl-glucosamine, GalNAc: *N*-acetyl-galactosamine.

**Table 2, part 3:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera (phyla)	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
<i>Coscinodiscus</i> (Oc)	<i>nobilis</i>	+	tr	-	+	+	-	++	+++	+	nd	+	Nd		[80]
<i>Cosmarium</i> (Cha)	sp.	+	++	+	+	nd	nd	+	+++	+	+	+	+		[52]
<i>Cryptocodinium</i> (Mi)	<i>cohnii</i>	nd	++	nd	+++	nd	nd	+	+	nd	+	nd	Nd		[81]
<i>Cylindrotheca</i> (Oc)	<i>closterium</i>	+	+	-	++	nd	nd	+	nd	+++	nd	-	+		[82]
	<i>fusiformis</i>	+	+++	-	++	nd	nd	+	+	+	-	+	Nd		[83]
<i>Cymbella</i> (Oc)	<i>cistula</i>	+	+++	tr	+	-	-	+	-	++	-	nd	Nd		[58]
	<i>mexicana</i>	+	+++	tr	+	tr	-	+	+	++	-	nd	Nd		[58]
<i>Desmococcus</i> (Chl)	<i>olivaceus</i>	+	+++	+	++	tr	tr	+	+	+	-	nd	+	linkages	[84]
<i>Dixonella</i> (Rh)	<i>grisea</i>	++	+	+	+	+	-	tr	tr	+++	-	nd	+	methyl groups	[91]
	<i>bardawil</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd		[48]
<i>Dunaliella</i> (Chl)	<i>tertiolecta</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd		[48]
	<i>salina</i>	-	+	-	+	-	-	-	-	+	-	nd	Nd		[43]
<i>Flintiella</i> (Rh)	<i>sanguinaria</i>	+	++	+	+	+	-	-	-	+++	-	tr	+	methyl and acetyl groups	[85]
<i>Geminella</i> (Chl)	<i>terricola</i>	++	+	+	+	nd	nd	+++	+	+	-	+	Nd		[56]
<i>Gyrodinium</i> (Mi)	<i>impudicum</i>	-	+++	-	-	+	+	-	-	-	-	+	+		[61]
<i>Haematococcus</i> (Chl)	<i>lacustris</i>	nd	++	nd	+	nd	nd	+++	nd	nd	nd	tr	Tr		[86]
<i>Heterosigma</i> (Chl)	<i>akashiwo</i>	++	+	+	+	+	-	+++	+	+	nd	+	Tr	ManA, linkages	[60]
<i>Hyalotheca</i> (Cha)	<i>dissiliens</i>	+	++	+	-	+++	-	-	+	+	-	nd	Nd	linkages	[55]
<i>Klebsormidium</i> (Cha)	<i>flaccidum</i> (749B)	++	+	+	+	nd	nd	+++	+	+	-	-	Nd		[56]

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Cha: Charophyta, Chl: Chlorophyta, Mi: Miozoa, Oc: Ochrophyta, Rh: Rhodophyta. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: N-acetyl-glucosamine, GalNAc: N-acetyl-galactosamine.

**Table 2, part 4:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera (phyla)	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
<i>Klebsormidium</i> (Cha)	<i>flaccidum</i> (446C)	+++	+	+	+	nd	nd	++	+	+	-	tr	nd		[56]
<i>Klebsormidium</i> (Cha)	<i>flaccidum</i> (748A)	+	+	+	tr	nd	nd	+++	++	+	-	+	nd		
<i>Melosira</i> (Oc)	<i>nummuloides</i>	+	tr	+	+	nd	nd	nd	+	+	nd	nd	nd		[65]
<i>Micrasterias</i> (Cha)	<i>denticulata</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		[87]
	<i>directa</i>	+	+	+	++	nd	nd	nd	+++	+	nd	nd	nd		[65]
<i>Navicula</i> (Oc)	<i>salinarum</i>	+	+	-	+++	nd	nd	+	nd	++	nd	+	+		[82]
	<i>subinflata</i>	+	++	+	+++	nd	nd	+	+	+	+	+	+	pyruvate	[88]
	<i>digitus</i>	+	+	+	+	nd	nd	+	+++	++	tr	+	+		
<i>Netrium</i> (Cha)	<i>interruptum</i>	+	+	+	+	nd	nd	+	++	+++	+	+	+		[52]
	<i>oblongum</i>	+	+++	+	+	nd	nd	+	+	++	tr	+	+		
<i>Oocystis</i> (Chl)	sp.	-	+	+	-	+	-	-	+	-	-	nd	nd		[77]
<i>Palmella</i> (Chl)	<i>mucosa</i>	-	-	+	+	+	-	-	+	-	-	nd	nd		
	<i>cylindrus</i>	+	+	+	+	nd	nd	+	+++	++	tr	+	nd		[52]
<i>Penium</i> (Cha)	<i>spirostriolatum</i>	+	++	+++	+	nd	nd	+	+	+	tr	+	+		
	<i>margaritaceum</i>	-	+	+	+	+	-	+	++	+++	-	nd	nd	methyl groups	[89]
<i>Pleurotaenium</i> (Cha)	<i>trabecula</i>	+	+	+	+	+	+	+	++	+++	+	+	+		[52]
<i>Porphyridium</i> (Rh)	<i>aerugineum</i>	-	++	-	+	+	-	-	-	+++	-	+	+	methyl groups	[50]

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Cha: Charophyta, Chl: Chlorophyta, Mi: Miozoa, Oc: Ochrophyta, Rh: Rhodophyta. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: *N*-acetyl-glucosamine, GalNAc: *N*-acetyl-galactosamine.

**Table 2, part 5:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera (phyla)	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
	<i>cruentum</i>	+	++	+	+	nd	nd	+	+	+++	-	+	+		[47]
	<i>cruentum</i>	-	+	-	++	+	-	-	-	+++	-	+	+	methyl groups	[50]
<i>Porphyridium</i> (Rh)	<i>marinum</i>	-	++	-	+	+	-	-	tr	+++	-	+	nd		[49]
	<i>purpureum</i>	-	+++	-	+	-	-	-	tr	++	-	+	-		[15]
	sp.	-	++	-	+	nd	nd	-	-	+++	-	+	nd	linkages	[90]
	<i>reticulata</i>	+	+	-	++	+	-	+	-	+++	+	nd	+		[92]
<i>Rhodella</i> (Rh)	<i>maculata</i>	+	+++	+	+	+	-	-	-	++	-	+	+		[15]
	<i>violacea</i>	++	+	+	+	+	-	-	-	+++	-	+	+		[25]
<i>Scenedesmus</i> (Chl)	<i>quadricauda</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd		[93]
<i>Spondylosium</i> (Cha)	<i>panduriforme</i>	tr	++	+	+	+	-	-	++	+	-	-	+	linkages	[53]
<i>Staurastrum</i> (Cha)	<i>iversenii</i>	+	++	-	-	-	-	-	+++	+	-	-	+		[94]
	<i>bacillaris</i> (772B ; 747C)	+++	+	+	+	nd	nd	+	tr	++	-	tr	nd		[56]
<i>Stichococcus</i> (Chl)	<i>bacillaris</i> (774E ; 677A)	+++	++	+	+	nd	nd	+	+	+	-	tr	nd		[56]
<i>Tetmemorus</i> (Cha)	<i>brebissonii</i>	+	++	+	+	nd	nd	+	+++	+	Tr	+	+	linkages	[52]
<i>Thalassiosira</i> (Oc)	sp.	++	+	-	+	+	-	+++	+	+	Nd	nd	+	GlcNAc, GalNAc, linkages	[95]

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Cha: Charophyta, Chl: Chlorophyta, Mi: Miozoa, Oc: Ochrophyta, Rh: Rhodophyta. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: *N*-acetyl-glucosamine, GalNAc: *N*-acetyl-galactosamine.

**Table 2, part 6:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
	<i>augstmalis</i>	tr	+	-	+++	-	++	+	+	-	-	nd	nd	GalN	[63]
	<i>cylindrica</i>	tr	+	-	+++	+	-	+	+	++	-	nd	nd	other uronic acid	[96]
	<i>flos-aquae</i>	-	-	-	+	+	-	-	-	+	+	nd	nd		[77]
<i>Anabaena</i>	sp.	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		[97]
	<i>spiroides</i>	++	+	nd	+	+++	-	+	+	+	nd	nd	nd	GlcNAc, GalNAc	[67]
	<i>sphaerica</i>	-	+++	tr	+	+	+	+	-	-	-	nd	nd		[98]
	<i>torulosa</i>	++	+	+	+	+	+	-	+	+++	-	nd	nd	GlcN	
<i>Anacystis</i>	<i>nidulans</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		[99]
<i>Aphanocapsa</i>	<i>halophytia</i>	+	+	-	++	-	-	+	+++	+	-	+	+		[100]
<i>Aphanothece</i>	<i>halophytica</i>	-	+	+	+++	+	-	+	++	-	-	nd	nd	linkages	[101]
	<i>maxima</i>	++	+	-	++	+	+	+	+	+++	-	nd	nd		[102]
<i>Arthrospira</i>	<i>platensis</i>	+	+++	tr	+	tr	+	tr	+	++	nd	tr	+		[103]
	<i>platensis</i>	+++	+	nd	+	++	++	tr	nd	+	nd	+	nd		[12]
	<i>pulvinata</i>	+	+	+	+++	nd	nd	+	+	+	-	tr	nd		[56]
<i>Calothrix</i>	sp.	-	++	+	+++	tr	+	+	+	+	-	nd	nd	GlcN	[63]
<i>Chlorogloeopsis</i>	sp.	+	+	+	++	+	+++	+	+	-	-	nd	nd		[98]

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: *N*-acetyl-glucosamine, GalNAc: *N*-acetyl-galactosamine.



**Table 2, part 7:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
<i>Chroococcus</i>	<i>minutus</i>	+	+	+	+++	+	tr	++	+	+	-	nd	+	GlcN, methyl groups	[104]
	<i>submarinus</i>	+	+	+	++	+	+	+	++	+++	+	+	+	GlcN	[64]
<i>Cyanospira</i>	<i>capsulata</i>	-	-	+	+	-	+++	+	+	-	-	-	+		[105]
<i>Cyanothece</i>	sp.	-	+	-	+++	+	+	++	+	+	-	-	tr		[106]
	sp.	-	-	-	+++	-	-	-	-	++	+	+	+		[62]
<i>Fischerella</i>	<i>musvicola</i>	-	+	-	+++	+	+	+	+	+	-	nd	nd		[98]
<i>Gloeocapsa</i>	<i>kuetzingigiana</i>	++	+	+++	+++	+	++	nd	+	+++	+	nd	nd	GalN, trace GlcN	[107]
	sp.	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		[13]
<i>Gloeocapsosis</i>	<i>crepidinum</i>	+	+	+	++	+	+	nd	+++	+	+	nd	nd	GlcN, GalN	[107]
<i>Gloeotheca</i>	sp.	+	++	-	+++	nd	nd	+	-	+	-	+	+	methyl groups	[108]
<i>Johannesbaptistia</i>	<i>pellucida</i>	+	+	+	+	+	+	+	+	+	-	+	+		[64]
	<i>foveolarum</i>	++	+	-	+++	nd	nd	+	-	+	-	nd	nd		[56]
<i>Leptolyngbya</i>	<i>tenuis</i>	++	+	-	+++	nd	nd	+	+	+	-	tr	nd		
	sp.	+	tr	tr	+++	+	++	nd	+	+	+	nd	nd	GalN, traces GlcN	[107]
<i>Lyngbya</i>	<i>conferviodes</i>	tr	++	tr	+++	+	nd	+	tr	tr	-	nd	nd		[109]
<i>Microcoleus</i>	<i>vaginatus</i>	+	++	+	+++	+	+	+	+	+	-	nd	+		[41]

+++ : first major monosaccharide, ++: 2nd major monosaccharide, +: presence, -: absence, tr: trace, nd: non determined. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: *N*-acetyl-glucosamine, GalNAc: *N*-acetyl-galactosamine.

**Table 2, part 8:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
<i>Microcoleus</i>	<i>vaginatus</i>	+	+	+	++	+	+	+++	+	+	-	nd	+	GlcNAc, linkages	[84]
	<i>aeruginosa</i>	++	+	nd	+	-	+	+	+	+++	nd	nd	nd	GlcNAc, GalNAc	[67]
<i>Microcystis</i>	<i>aeruginosa</i>	+++	-	-	+	nd	nd	++	-	-	-	-	-		
	<i>aeruginosa flos-aquae</i>	-	-	-	+++	nd	nd	++	-	-	-	-	-		[110]
	<i>viridis</i>	++	-	-	+++	nd	nd	+	+	-	-	nd	nd		
	<i>caldicola</i>	+	+	+	+++	+	+	+	+	++	-	nd	+		[73]
	<i>carneum</i>	-	-	-	-	-	-	+++	-	++	-	-	+		[62]
	<i>commune</i>	-	++	-	+++	-	nd	+	-	-	nd	nd	nd		[111]
	<i>flagelliforme</i>	-	++	+	+	+	nd	tr	+	+++	nd	nd	nd		
	sp.	-	+	+	+	-	-	+	-	-	-	nd	nd		[77]
<i>Nostoc</i>	sp.	+	++	-	+++	+	+	+	-	+	-	nd	+		[40]
	<i>insulare</i>	-	-	++	+++	+	-	-	-	-	-	-	tr	trace acetate, linkages	[112]
	<i>insulare</i>	tr	tr	+	+++	++	+	+	+	tr	-	nd	+		[104]
	<i>muscoru</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		[13]
	<i>entophytum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		
	sp.	+	+	+	+++	+	+	+	+	++	-	nd	nd		[63]
<i>Oscillatoria</i>	<i>amphibia</i>	+	+	tr	+++	nd	nd	++	tr	+	-	nd	nd		[109]

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: *N*-acetyl-glucosamine, GalNAc: *N*-acetyl-galactosamine.

**Table 2, part 9:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
	<i>corallinae</i>	+	+	+	+++	+	nd	++	+	+	-	nd	nd		[109]
<i>Oscillatoria</i>	sp.	tr	-	-	+++	-	-	-	tr	tr	-	-	+	other uronic acid	[64]
	sp.	-	+	+	++	+	+++	-	+	+	-	nd	nd		[98]
	sp.	-	-	-	+++	-	-	-	-	++	+	-	+		[62]
	<i>autumnale</i>	+++	tr	-	+	-	+	+	tr	tr	+	nd	nd		[63]
	<i>battersii</i>	-	++	+	+++	+	+	++	+	+	-	+	+		[64]
	<i>ambiguum</i>	+++	++	+	++	+	+	+	+	+	-	tr	nd		
	<i>corium</i> (442D & 746B)	++	+	+	+++	nd	nd	+	+	+	-	tr	nd		
	<i>corium</i> (444A)	+	+++	+	++	nd	nd	+	+	+	-	tr	nd		[56]
	<i>corium</i> (674A)	++	+	-	+	nd	nd	+	-	+++	-	nd	nd		
<i>Phormidium</i>	<i>corium</i> (743D)	+	+	-	+++	nd	nd	++	-	+	-	nd	nd		
	<i>ectocarpi</i> (K5)	+	++	tr	+++	+	nd	+	tr	+	-	nd	nd		
	<i>ectocarpi</i> (ME3)	+	+	tr	+++	+	nd	++	tr	+	-	nd	nd		
	<i>ectocarpi</i> (N182 ; C86)	-	+	-	+++	+	nd	++	-	+	-	nd	nd		[109]
	<i>foveolarum</i> (C52)	+	+	tr	+++	+	nd	++	tr	+	-	nd	nd		
	<i>foveolarum</i> (MEU)	+	++	+	+++	-	-	+	+	+	-	nd	nd		
	<i>minutum</i> (D5)	tr	+++	-	++	nd	nd	+	+	+	-	nd	nd		

+++ : first major monosaccharide, ++ : 2nd major monosaccharide, + : presence, - : absence, tr : trace, nd : non determined. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: N-acetyl-glucosamine, GalNAc: N-acetyl-galactosamine.


**Table 2, part 10:** Monosaccharide and non sugars groups compositions of EPS from microalgae/cyanobacteria described in current literature.

Genera	Species	Rha	Gal	Ara	Glc	GlcA	GalA	Man	Fuc	Xyl	Rib	SO <sub>4</sub> <sup>2-</sup>	Proteins	Other informations	References
<i>Phormidium</i>	<i>minutum</i> (NB5)	tr	+	+	+++	nd	nd	++	-	+	-	nd	nd		
	<i>minutum</i> (RT6)	+	+	++	+++	+	nd	+	+	+	-	nd	nd		
	sp. (CCAP1464/3 ; CCAP1463/4)	+	++	+	+++	+	nd	+	tr	+	-	nd	nd		[109]
	sp. (PNG91 ; 90-14/1)	+	+	+	+++	nd	nd	++	+	+	-	nd	nd		
	sp.	+	+	+	+++	+	+	-	+	++	-	nd	nd		[98]
<i>Plectonema</i>	<i>tenue</i>	+	+	+++	++	nd	tr	+	+	+	-	nd	+	GlcNAc, linkages	[84]
	<i>usteri</i>	+	+++	+	++	nd	nd	+	+	+	-	tr	nd		[56]
	<i>golenkinianum</i>	-	-	-	+++	-	-	-	tr	tr	-	-	+		[64]
<i>Rhabdoderma</i>	sp.	+	++	nd	+++	tr	+	nd	tr	+	+	nd	nd	GlcN, trace GalN	[107]
	<i>rubrum</i>	-	+	-	+	tr	tr	+	+	++	-	+	+	GlcN +++	[64]
<i>Scytonema</i>	<i>hofmanni</i>	-	+	-	+	+	+	+++	-	-	-	nd	nd		[98]
	<i>javanicum</i>	+	++	+	+++	tr	tr	+	tr	+	-	nd	+	linkages	[84]
<i>Synechococcus</i>	sp.	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		[36]
	<i>aquatilis</i>	+	+	-	+++	+	+	+	+	+	-	nd	nd	GlcN ++	[63]
<i>Synechocystis</i>	sp.	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		[13]
	sp. (PCC6803)	+	+	-	+++	+	+	+	++	+	-	+	+	GlcN, GalN	
	sp. (PCC6714)	+	++	+	+++	+	+	++	+	+	-	+	+	GlcN, GalN	[113]
<i>Tolypothrix</i>	<i>tenuis</i>	+	+	+	+++	+	+	++	+	-	-	nd	nd	GlcN	[98]
<i>Trichormus</i>	<i>variabilis</i> (VRUC162)	tr	+	tr	+++	-	-	+	+	++	+	nd	nd	GalN	
	<i>variabilis</i> (VRUC168)	+	+	+	+++	+	+	+	+	++	-	nd	nd	GlcN	[63]

+++; first major monosaccharide, ++; 2nd major monosaccharide, +; presence, -; absence, tr: trace, nd: non determined. Rha: rhamnose, Gal: galactose, Ara: arabinose, Glc: glucose, Man: mannose, Fuc: fucose, Xyl: xylose, Rib: ribose, GlcA: glucuronic acid, GalA: galacturonic acid, GlcN: glucosamine, GalN: galactosamine, ManA: mannuronic acid, GlcNAc: *N*-acetyl-glucosamine, GalNAc: *N*-acetyl-galactosamine.

**Table 3:** Monosaccharides compositions (molar ratios) of EPS from strains EPS+ obtained by GC/MS and HPAEC-PAD.

Genera	Species	RCC numbers	Rha		Gal		Ara		Glc		Man		Fuc		Xyl		Rib		GlcA		GalA	
			(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
<i>Chrysothila</i>	<i>dentata</i>	CCAP 904/1	-	-	33	36	36	33	tr	tr	10	8	-	tr	17	16	-	-	4	4	-	tr
	sp.	4633	tr	tr	31	33	13	11	42	45	-	-	-	-	10	8	-	-	4	2	-	tr
<i>Isochrysis</i>	<i>braarudii</i>	3686	9	4	27	23	39	47	3	3	-	-	-	-	18	19	+	+	4	2	-	-
		6688	15	19	36	46	4	tr	-	-	34	32	-	-	-	-	-	-	11	tr	-	tr
<i>Diacronema</i>	sp.	3514	33	37	26	23	17	8	15	16	-	-	-	-	5	10	-	-	4	4	-	tr
<i>Exanthemachrysis</i>	sp.	1544	2	3	16	14	8	14	8	7	15	13	-	-	14	12	-	-	37	32	-	5
<i>Pavlova</i>	<i>gyrans</i>	1545	3	4	32	21	13	18	14	7	-	-	tr	6	21	25	-	-	9	11	8	7
	sp.	4037	46	36	10	11	26	23	6	10	-	-	-	2	12	14	-	-	-	2	-	2
	sp.	4120	7	7	34	40	13	4	23	35	4	-	-	7	19	2	-	-	tr	3	-	2
	sp.	3438	47	30	27	29	5	14	11	17	tr	-	-	-	3	6	-	-	5	4	-	-
<i>Glossomastix</i>	sp.	3688	28	20	4	2	-	-	-	-	-	-	54	57	-	-	-	-	9	10	5	11
	sp.	3685	20	19	-	-	-	-	-	-	-	-	56	57	-	-	-	-	10	8	14	13
	sp.	3707	31	27	2	3	-	-	-	tr	-	-	40	47	-	tr	-	-	6	7	21	15
<i>Synechococcus</i>	sp.	2380	-	tr	38	27	-	tr	38	49	-	-	24	20	-	-	-	-	-	tr	-	tr
<i>Tetraselmis</i>	<i>globosa</i>	1564	63	61	19	20	tr	tr	5	5	-	-	-	-	5	4	-	-	-	2	5	-
	<i>rubens</i>	133	nd	6	nd	89	nd	-	nd	tr	nd	-	nd	2	nd	tr	nd	-	nd	-	nd	2
	sp.	4435	5	3	33	35	24	19	5	7	8	7	-	9	23	14	-	-	2	4	-	2
<i>Dunaliella</i>	sp.	5	18	21	19	28	4	5	2	tr	11	13	-	-	13	17	-	-	19	4	14	11
<i>Prasinococcus</i>	sp.	2684	7	4	67	74	7	3	9	7	-	-	-	2	7	9	-	-	3	tr	-	-
<i>Porphyridium</i>	sp.	4628	-	-	29	27	2	2	22	20	-	-	-	-	44	49	-	-	3	2	-	-

(1): GC/MS analyses, (2): HPAEC-PAD analyses, +: presence, -: absence, tr: trace, nd: non determined,  no classification available. The SD was < to 5 % for all experiments.

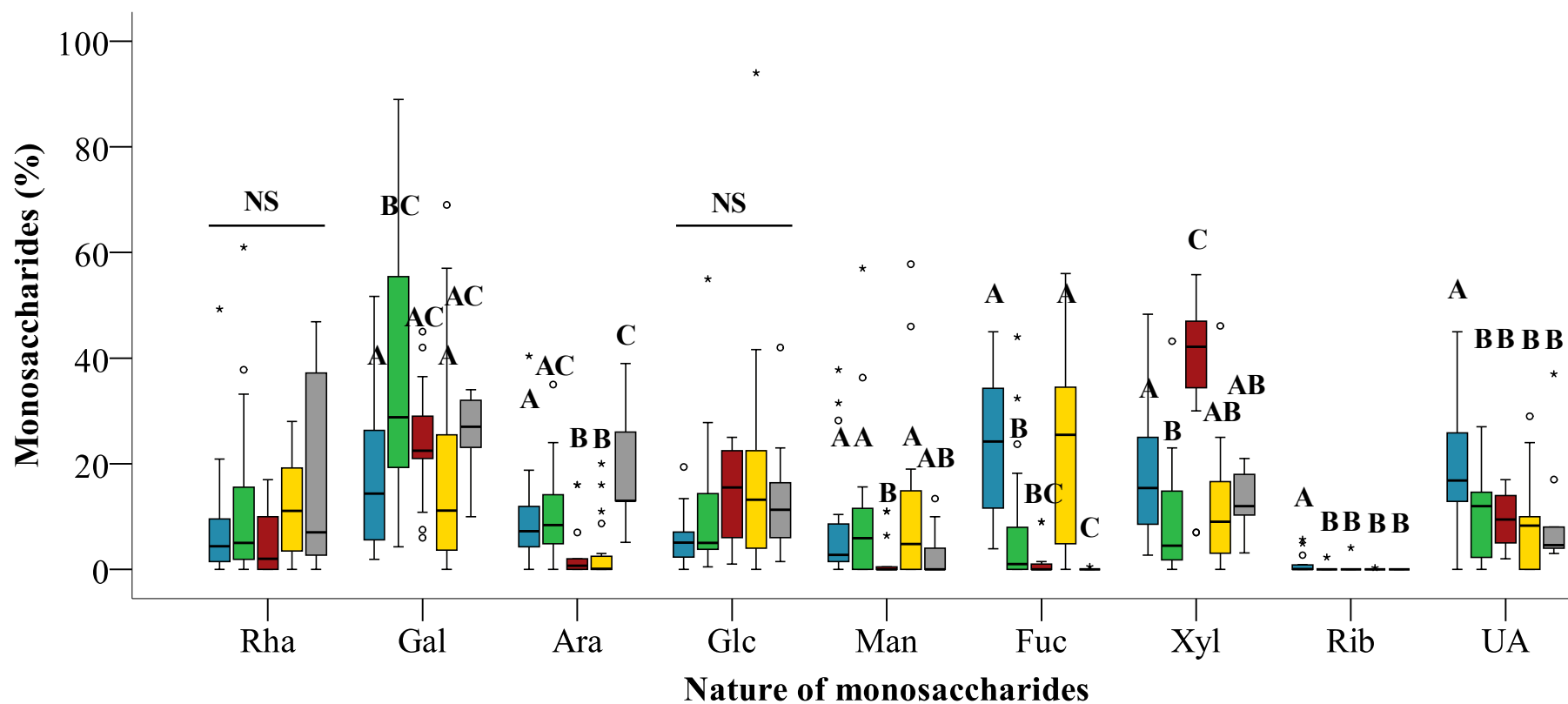
EPS from *Porphyridium* sp. (RCC4628) had also a composition very similar to those already determined in the literature on different species of *Porphyridium* including *aeruginum*, *marinum*, *cruentum* and *purpureum* [15, 47, 49-50]. It was a heteroxylan composed of Xyl, Gal and Glc (49, 27 and 20 %, respectively) and other minor monosaccharides. Philips et al. [36] studied the physico-chemical properties of solutions of an EPS from a *Synechococcus* strain for which no monosaccharides composition was determined. Results obtained in this study revealed that the EPS from *Synechococcus* sp. (RCC2380) was composed of Glc, Gal and Fuc (49, 27 and 20 %, respectively).

This study also described for the first time the composition of EPS i) in species belonging to the Haptophyta phylum (*Chrysolita* (CCAP904/1, RCC4633), *Diacronema* (RCC3514), *Exanthemachrysis* (RCC1544), *Isochrysis* (RCC3686), *Pavlova* (RCC1545, RCC3438, RCC4120, RCC4037), ii) in two classes belonging to the Chlorophyta (Chlorodendrophyceae represented by the genus *Tetraselmis* (RCC133, RCC1564 and RCC4435) and Palmophyllophyceae with *Prasinococcus* sp. (RCC2684)), and iii) in the genus *Glossomastix* (RCC3685, RCC3688 and RCC3707) (Pinguiphyceae, Ochrophyta phylum). The compositions were extremely variable even if all EPS were heteropolysaccharides. They are mainly composed of three or five monosaccharides including neutral sugars and uronic acids apart for the biopolymers produced by *Pavlova* sp. (RCC4120) and *Tetraselmis globosa* (RCC1564) mainly composed of two monosaccharides representing 81 and 75 % of the total monosaccharides, respectively. EPS of *Tetraselmis rubens* (RCC133) and *Prasinococcus* sp. (RCC2684) are galactans since Gal represents 74 and 89 % of the total monosaccharides compositions, respectively. **Table 3** shows that all the neutral sugars usually detected in the composition of microalgae/cyanobacteria EPS (Fuc, Ara, Rha, Gal, Glc, Gal, Xyl and Man) were present in the composition of biopolymers analyzed in this study. After analysis of the 17 EPS compositions, Gal, Rha, Xyl, Fuc, Ara, Glc and Man were respectively identified as the main

monosaccharide in 7, 4, 2, 2, 1, 0 and 0 EPS (meaning for example Gal was the main monosaccharide in 7 EPS compositions). Similarly, Gal, Rha, Xyl, Fuc, Ara, Glc and Man were quantitatively the second monosaccharide in 6, 2, 2, 0, 3, 1 and 1 EPS compositions. The EPS of *Exanthemachrysis* sp. (RCC1544) was distinguished by its richness in GlcA representing around 35 % of its composition. Note that PS mainly composed of glucuronic acids and called glucuronans are poorly detected in natural biomass and are very attractive for their biological and rheological properties [51]. In addition, most of the EPS identified in this study (with the exception of *Synechococcus* sp. (RCC2380)) contained at least one uronic acid (GlcA or GalA) and most of the time both. They are present in variable content, sometimes in trace amounts (EPS from RCC6688), in small quantities (EPS from *Tetraselmis* sp. (RCC4435) and *Pavlova* sp. (RCC4120 and RCC4037)) or in very large quantities as previously described in EPS from *Exanthemachrysis* sp. (RCC1544) and to a lesser degree in that of *Pavlova gyrans* with 35 % and 11 % of GlcA, respectively. Overall, this work confirmed that EPS from microalgae have very complex compositions, probably evolved in response to biotic and/or abiotic conditions.

### **3.3. Relationship between phylogeny and exopolysaccharides compositions**

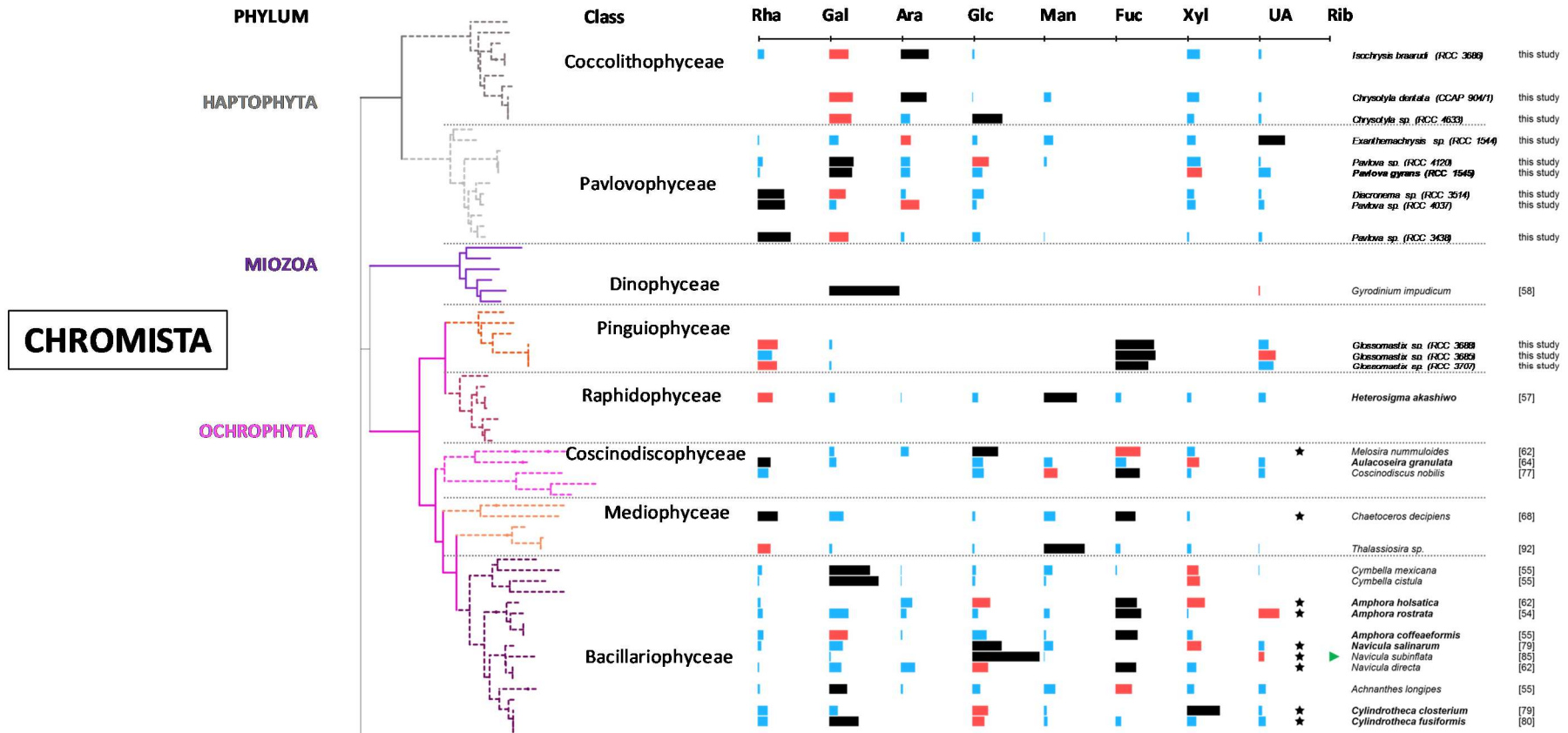
We performed a metanalysis on the EPS compositions of 81 strains of microalgae from bibliographic data and experimental results obtained in this study in order to determine whether they could vary according to their taxonomic affiliation. This metanalysis was not done on the composition of EPS from cyanobacteria as their specificity is the systematic presence of Glc as main (63 %) or second monosaccharides (18 %) (**Table 2**). Levels of some monosaccharides varied significantly between phyla ( $p < 0.05$  in Kruskal-Wallis tests for Gal, Ara, Man, Fuc, Xyl, Rib and Uronic acids) while Rha and Glc levels were relatively constant across phyla (**Figure 1**,  $p = 0.117$  and  $p = 0.058$ , respectively). Such differences appear also on the phylogenetic tree presented on **Figure 2**.



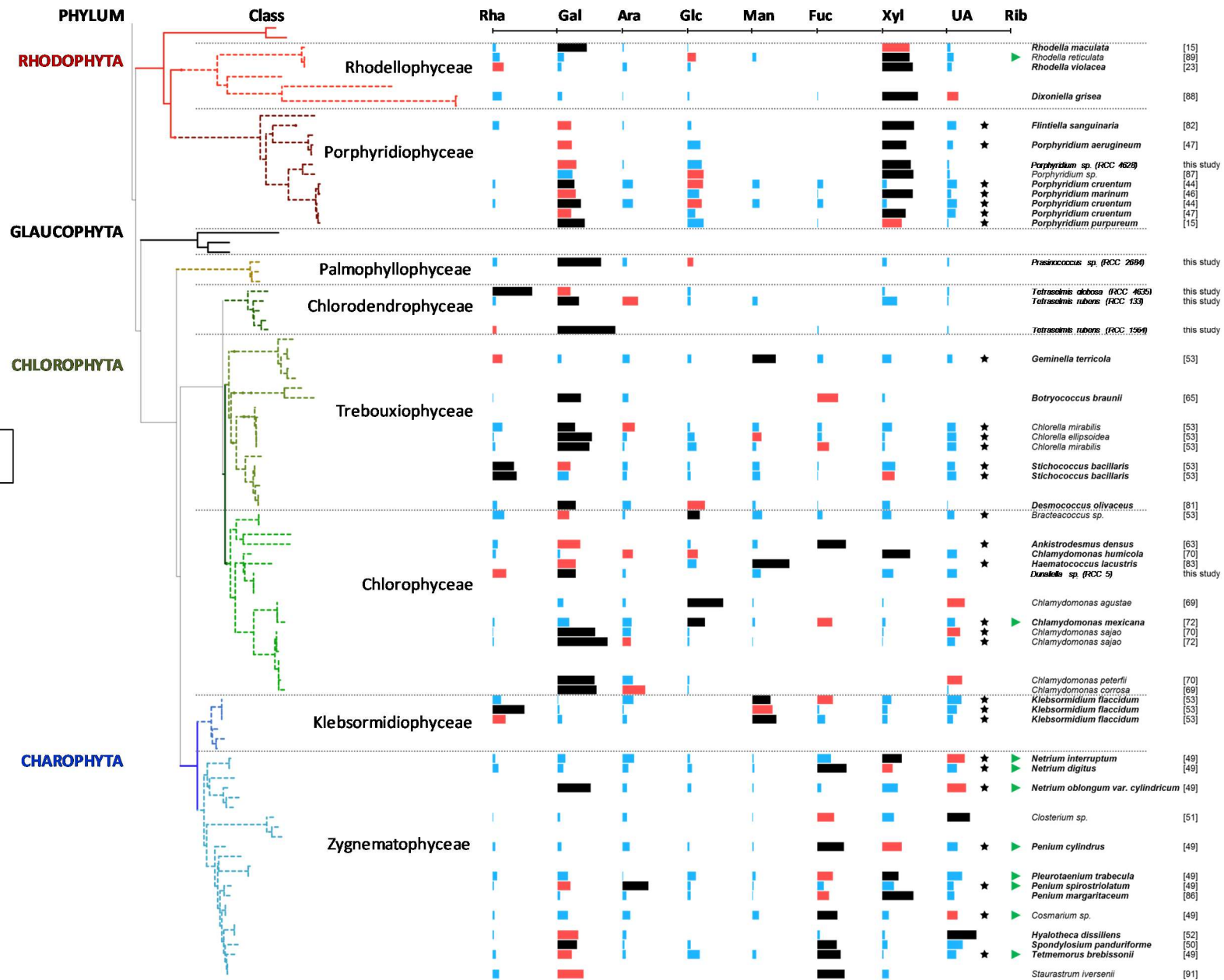
**Figure 1:** Variations in monosaccharides (Rha (rhamnose), Gal (galactose), Ara (arabinose), Glc (glucose), Man (mannose), Fuc (fucose), Xyl (xylose), Rib (ribose)) and uronic acids (UA), given in % of the total composition) in EPS of Charophyta (■), Chlorophyta (■), Rhodophyta (■), Ochrophyta (■) and Haptophyta (■) (16, 23, 13, 20 and 9 monosaccharides compositions, respectively). Data obtained from the literature and those included in this study are shown. Miozoa were not included in this analysis, since only two monosaccharide compositions were available. Miozoa were not included in this analysis, since only two monosaccharides compositions were available. Results are given as box-plots such as the central



lines represent the medians, boxes the lower 25 % to the upper 75 % quartile, and the whiskers the range excluding outliers, that are given as open circles and stars. For each monosaccharide, Kruskal-Wallis tests were performed. When a significant difference was found ( $p < 0.05$ ), this test was followed by multiple pairwise comparisons (Mann-Whitney tests), with significance levels adjusted according to the Benjamini and Hochberg method (1995) [28]. Different letters indicate statistically significant differences between phyla. NS: Non-Significant results. The classification was done according to the AlgaeBase database [29]. Different letters indicate statistically significant differences between phyla. ○ and ★: outliers. NS: Non-Significant.



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**Figure 2, parts 1 and 2:** Composition in neutral sugars (Rha (rhamnose), Gal (galactose), Ara (arabinose), Glc (glucose), Man (mannose), Fuc (fucose), Xyl (xylose), Rib (ribose)) and uronic acids (UA) (in % of the total composition 0-100 % scale) of EPS from 81 microalgae (16 Charophyta, 23 Chlorophyta, 13 Rhodophyta, 20 Ochrophyta and 9 Haptophyta) placed on a Neighbor-Joining phylogenetic tree based on 18S rDNA sequences. ■: 1<sup>st</sup> major monosaccharide, ■: 2<sup>nd</sup> major monosaccharide, ■: secondary monosaccharides, ★: % of UA was obtained by colorimetric assay, ►: presence of Rib. The positions of the strains written in bold were determined from sequences corresponding to the right species. For the other species, sequences from a representative of the same genus were used. The classification was done according to the AlgaeBase database [29].

Clear differences, between the 3 phyla of the plantae kingdom included in the analysis (Charophyta, Chlorophyta and Rhodophyta), have been detected. EPS from Charophyta have the most distinct composition profile, with higher Fuc levels compared to Chlorophyta and Rhodophyta. Fuc was the first or second monosaccharide in 10/16 profiles, while it was only 4/23 profiles of the Chlorophyta and none in the Rhodophyta ( $p < 0.001$  for each comparison). They also contain significantly higher levels of uronic acids compared to EPS from the two other Plantae phyla ( $p = 0.013$  and  $p = 0.01$  when compared to Chlorophyta and Rhodophyta, respectively). They can represent up to 28 and 29 % in EPS of *Netrium interruptum* and *Netrium oblongum*, respectively [52]. In EPS from *Spondylosium panduriform*, uronic acids were only represented by GlcA which represents 24 % of the total composition [53], and it is even the major monosaccharide in EPS from *Closterium* sp. and *Hyalotheca dissiliens* [54-55]. EPS from Charophyta were also characterized by the presence of Rib (8/16 profiles) which contrasts with those of Chlorophyta and Rhodophyta profiles (1/23 and 1/13, respectively). This characteristic seems to be specific of the Zygnematophyceae (8/13 profiles) since Rib was not found in Klebsormidiophyceae. More specifically, it is systematically present in traces in *Netrium*, *Cosmarium*, *Penium*, *Tetmemorus*, *Pleurotaenium* genus [52]. A large majority of Charophyta EPS contain sulphate groups, except EPS from *Klebsormidium flaccidum* (749B) [56]. The sulphate content of some EPS can reach up  $> 10$  % as for those of *Tetmemorus brebissonii* and *Pleurotaenium trabecula* [52].

EPS from Rhodophyta are characterized by high levels of Xyl ( $p = 0.005$  and  $p = 0.001$  when compared to Charophyta and Chlorophyta, respectively). This monosaccharide is the first or second in 11/13 profiles, which contrasts with those from Chlorophyta (2/23) and Charophyta (5/16). The Porphyridiaceae family (including *Porphyridium* and *Flintiella* genera) was characterized by the production of galactoxylans, whereas EPS from the Glaucosphaeraceae

family were rhamnoxylans and/or galactoxylans, depending on the species. All these EPS were sulphated (excepted for *Flintiella sanguinaria*), sometimes with a content > 10 % [49-50].

High levels of Gal were often observed in EPS from Chlorophyta (this monosaccharide being the first or second in 18/23 profiles) which differs from Charophyta ( $p = 0.025$ ) but not from Rhodophyta ( $p = 0.15$ ), which have also Gal as a first or second monosaccharide in 9/13 profiles.

The second kingdom analysed is the Chromista. Results presented part 3.2 have highlighted Haptophyta producing EPS. Thus, there are 3 phyla involved in EPS production, including Ochrophyta, Haptophyta, and Miozoa phyla. As only two monosaccharide compositions were available for Miozoa, comparison analyses were only performed on Ochrophyta and Haptophyta. The main differences between Ochrophyta and Haptophyta lie in the differences in Fuc contents ( $p < 0.001$ ) (**Figure 2**). Indeed, Fuc is present in most EPS of Ochrophyta (17/20 profiles), as the first or second monosaccharide (11/20 profiles) whereas it is present in only 2 species of Haptophyta on the 10 analyzed. In the genera *Amphora* and *Glossomastix* in particular, Fuc represents respectively more than 30 and 46 % of the total monosaccharide composition [57-58]. Differences in Ara levels can also be observed ( $p < 0.001$ ). While most of the Ochrophyta EPS contain, at best, traces of Ara (content > 3 % of the composition in only 4/20 profiles), this monosaccharide is present at significantly higher levels in Haptophyta (content > 7 % in 8/10 profiles).

Although the analysis was focused on the distinctions of EPS compositions between phyla, it was contested that in the Chromista some species belonging to the same genus had very varied compositions, as *Pavlova* species for example. These differences could be explained by the fact that *Pavlova* is a polyphyletic genus [59]. Specific composition was found for the EPS of *Heterosigma akashiwo* which was the sole example of microalgae EPS containing

ManA [60]. The composition of *Gyrodinium impudicum* EPS is also special because this is a homopolysaccharide: a sulphated galactan [61].

Finally, the last kingdom and also phylum is the cyanobacteria. EPS production by these photosynthetic microorganisms has been abundantly published, maybe because of their ecological abundance and easy growing. All these data make it possible to view the large diversity of composition of the exopolymers resulting from this phylum (**Table 2**). The main characteristic of cyanobacteria is the systematic presence of Glc, with the exception of *Nostoc carneum* [62]. It is the principal or second monosaccharide in 65 (50/78 profiles) and 18 % (14/78 profiles) EPS, respectively. In comparison, Xyl appearing as main monosaccharide just after Glc is 7 times less present (7/78 profiles). Some specific compositions have been highlighted. Osamines (GlcN and or GalN) were found in 21 compositions. In addition, GlcN is the first and second monosaccharide in EPS of *Rhabdoderma rubrum* and *Synechocystis aquatilis*, respectively [63-64]. Moreover, similar EPS compositions were found between strains belonging to the same genus, as *Synechocystis* [98, 113], *Phormidium* [109], *Oscillatoria* [64, 98, 109], *Leptolyngbya* [56, 107] and *Plectonema* [56, 64, 107].

#### 4. Conclusions

One hundred and sixty-six microalgae and cyanobacteria were screened and 45 of them were detected as producers of a soluble EPS based on colorimetric assays. The composition analyses performed on 20 of them confirmed they were EPS. This allowed to highlight 8 new genera of microalgae and to characterize 17 new monosaccharide compositions. Overall, all the microalgae and cyanobacteria EPS are heteropolymers. They are produced by 49 and 29 genera in microalgae and cyanobacteria, respectively, which represent 134 species. Some common points in composition can be established according to the phylogenetic affiliation.

EPS from Charophyta contain high Fuc and uronic acids levels and were characterized by the presence of Rib in the half part on EPS compositions available. Moreover, a large

majority of their EPS contain sulphate groups. EPS from Rhodophyta were characterized by high levels of Xyl, and the Porphyridiaceae and Glaucosphaeraceae families produce compositions that were specific to them. High levels of Gal were often observed in EPS from Chlorophyta. In Chromista, Fuc is present in most EPS of Ochrophyta compare to Haptophyta but Ara levels were significantly higher in Haptophyta compare to EPS of Ochrophyta. To conclude, EPS produced by cyanobacteria were mainly composed Glc, and this monosaccharide is always present.

Thanks to the growing number of studies and the 132 compositions available, the metanalysis on the EPS compositions by statistical tests made it possible to distinguish specific compositions between phyla. This finding is clearly the proof that evolution has conserved some complex biosynthesis pathways inside specific phyla to give an advantage to strains having the ability to produce some exopolysaccharides in some environments. However, the current lack of knowledge on the physiological function of EPS despite the abundance of literature on this subject makes it difficult to correlate some polysaccharidic structures with their biological roles. Nevertheless, there are still a lot of microalgae to study and only a tiny part of the phylogenetic tree has been concerned (insufficient data on Miozoa phylum for example).

### **Conflict of interest**

The authors declare that they have no conflict of interest.

### **Authors' contributions**

Philippe Michaud conceived the project and reviewed the manuscript. Céline Laroche designed the experiments for microalgae screening and culture in PBR; Guillaume Pierre, Cedric Delattre and Pascal Dubessay have setup methods for sugars analysis and molecular biology. Clément Gaignard has done the screening of microalgae and polysaccharides analysis, interpreted the results and wrote the manuscript. Christine Gardarin realised analysis by ionic chromatography;



Aurore Dubuffet conducted the phylogenetic analysis and interpreted the results; Priscillia Gourvil and Ian Probert have chosen the microalgae strains considering their phylogeny and phenotypes. All authors approved the final manuscript.

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### **Statement of informed consent, human/animal rights**

No informed consent, human or animal rights applicable

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