



Potential valorization of polymeric substances excreted by *Chaetomorpha aerea*, a macro-algae harmful to French oyster industry

Guillaume Pierre, Valérie Sopena, Camille Juin, Amira Mastouri, Marianne Graber, Thierry Maugard

► To cite this version:

Guillaume Pierre, Valérie Sopena, Camille Juin, Amira Mastouri, Marianne Graber, et al.. Potential valorization of polymeric substances excreted by *Chaetomorpha aerea*, a macro-algae harmful to French oyster industry. *Biotechnology and Bioprocess Engineering*, 2011, 16, pp.937-945. hal-00652073

HAL Id: hal-00652073

<https://hal.science/hal-00652073>

Submitted on 15 Dec 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Antibacterial activity of a sulfated galactan extracted from the marine alga *Chaetomorpha aerea* against *Staphylococcus aureus*.

Guillaume Pierre¹, Valérie Sopena¹, Camille Juin¹, Amira Mastouri¹, Marianne Graber¹, Thierry Maugard^{1,*}

(1) UMR 6250 CNRS - ULR LIENSs. Université de La Rochelle, UFR Sciences, Bâtiment Marie Curie, avenue Michel Crépeau, 17042 La Rochelle, France.

* Tel: (33) 5 46 45 82 77 Fax: (33) 5 46 45 82 65 E-mail: thierry.maugard@univ-lr.fr

Abstract

The *in vitro* antimicrobial activity of the marine green algae *Chaetomorpha aerea* was investigated against gram-positive bacteria, gram-negative bacteria, and a fungus. The water-soluble extract of algae was composed of a sulfated (6.3%) galactan with a molecular weight of 1.160×10^6 Da and a global composition close to commercial polysaccharides as dextran sulfate or fucoidan. The polysaccharide was composed of 18% arabinose, 24% glucose, 58% galactose. The re-suspended extracts (methanol, water) exhibited selective antibacterial activities against three gram-positive bacteria including *Staphylococcus aureus* (ATCC 25923). Minimum inhibitory concentration and minimum bactericidal concentration tests showed that the sulfated galactan could be a bactericidal agent for this strain ($40 \text{ mg} \cdot \text{mL}^{-1}$). Results of the present study confirmed the potential use of the green algae *Chaetomorpha aerea* as a source of antibacterial compounds or active known molecules.

Keywords: Seaweed, *Chaetomorpha aerea*, sulfated galactan, antibacterial activity, *Staphylococcus aureus*

INTRODUCTION

Seaweeds are used by coastal populations for thousands of years owing to their high nutritional values [1, 2]. However, the industrialization of seaweeds does not necessarily need their consumption. Medical and pharmaceutical industries are also interested since marine plants are rich in active molecules [3, 4]. Indeed, the therapeutic potentials of certain substances are extremely promising, especially as antimicrobial and antiviral factors [5, 6]. Besides, the use of fucoidans allows fighting against the formation and growth of malignant tumors [7, 8]. Numerous studies have investigated the biological activities of algae extracts [9]. Different seaweeds, e.g. *Ulva fasciata* or *Enteromorpha compressa*, have presented antimicrobial activities against *Staphylococcus aureus* or *Pseudomonas aeruginosa*, two bacteria commonly found in many human infections [10]. Nevertheless, microorganisms are able to adapt their metabolism for resisting to the action of antimicrobial drugs [11, 12]. This problem is one of the main reasons which require further research of new antimicrobial compounds, including molecules from marine algae [13]. *Chaetomorpha aerea* is a green filamentous alga which develops in many marine mediums as oyster ponds. Numerous species of *Chaetomorpha* have often been studied since these organisms behave like opportunistic macrophytes, causing evident ecological changes in the ecosystem contaminated. In French oyster ponds (Marennes-Oléron, France), the excessive proliferation of these seaweeds prevent the proper development of the phytoplanktonic portion that oysters need to grow up. More generally, different studies have attempted to positively exploit these algae. It was reported that *Chaetomorpha linum* was able to chelate heavy metals (copper and zinc) in aqueous solutions [14]. A heparin-like polysaccharide has been highlighted in the seaweed *Chaetomorpha antennina* [15]. Finally, the biological properties of sulfated arabinogalactans [16, 17] extracted from green algae like *Chaetomorpha* were investigated. In this way, an

interesting polysaccharide composed of arabinose (57%), galactose (38.5%), rhamnose (3.8%) and sulfates (11.9%) was purified from *Chaetomorpha linum* [18].

In the present study, we have developed a method to extract extracellular polysaccharides from *Chaetomorpha aerea*. These polysaccharides were studied and biochemically characterized to determine their composition and their partial structure. We have also investigated the potential antimicrobial activities of these different extracts against microorganisms, i.e. *Staphylococcus aureus* (ATCC 25923), *Salmonella enteritidis* (ATCC 13076), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (CIP 103214), *Bacillus subtilis* (CIP 5262), *Micrococcus luteus* (ATCC 4698) and *Candida glabrata* (DSMZ 6425). This investigation could scientifically proof that the natural compounds of *Chaetomorpha aerea* could be potentially used as antibacterial agents.

MATERIALS AND METHODS

Materials

Ground and dried *Chaetomorpha aerea* (Fig. 1), harvested on oyster ponds (Marennes-Oléron, France) during winter (January 2009). Dowex Marathon C, BicinChonic Acid (BCA) Protein Assay Kit, Azure A, N,O-bis(trimethylsilyl)trifluoroacetamide: trimethylchlorosilane (BSTFA: TMCS) (99: 1), Zinc sulfate and Baryum hydroxide were obtained from Sigma–Aldrich. Standard carbohydrates (dextran, dextran sulfate, blue dextran, heparin, fucoidan, glucose, galactose, rhamnose, fucose, fructose, xylose, arabinose, mannose, lactose, raffinose, myo-inositol, glucuronic and galacturonic acid) and a protein standard (Bovine Serum Albumin, BSA) were obtained from Sigma–Aldrich. Fucogel 1000PP, composed of a 3)- α -L-Fucp-(1 \rightarrow 3)- α -D-Galp-(1 \rightarrow 3)- α -D-GalpA-(1 \rightarrow polysaccharide, was obtained from Solabia [19]. Solvents (chloroform, hexane, ethanol) were from Carlo Erba. The ICSep ORH-801 and TSK Gel G3000 PWXL-CP columns for High

Performance Liquid Chromatography analysis (HPLC) were obtained from Interchim. The DB-1701 J&W Scientific column (30m, 0.32mm, 1mm) for Gas Chromatography-Mass Spectrometry analysis (GC/MS) was obtained from Agilent.

Extraction and purification methodology

From 5g of ground and dried *Chaetomorpha aerea*, a first step of delipidation and depigmentation was performed by using mixtures of chloroform/hexane (2/1 v/v) (Fig. 2, Step 1). The second step consisted to extract the extracellular polymers through an aqueous extraction procedure, during 24h at 40°C (Fig. 2, Step 2). A filtration stage was then applied to clear out unwanted residues. After freeze-drying, the combination of aqueous solutions of zinc sulfate (5%) and barium hydroxide (0.3N) on the sample allowed realizing the defecation step (Fig. 2, Step 3). The supernatant was recovered after centrifugation then freeze-dried. Finally, a purification step was applied to the sample through dialysis, to further purify the carbohydrate fraction (Fig. 2, Step 4). This fraction was named *Chaetomorpha aerea* carbohydrates-rich (CACR) fraction. A simple extraction was done (only the step 2) to compare the composition of the CACR fraction with this water-extracted fraction. This fraction was named control fraction (CF).

Biochemical characterization

Total sugar content was determined using the phenol-sulfuric acid assay, developed by Dubois, using glucose as a standard [20]. Protein content was determined using the bicinchoninic acid (BCA) assay, using bovine serum albumin (BSA) as a standard [21]. The sulfate content was measured by the Azure A [22] and the barium chloride gelation method [23], using dextran sulfate as a standard. Fourier Transform Infrared Spectroscopy (FTIR) analyses were performed on the CACR fraction and commercial controls (dextran, dextran sulfate, fucoidan, bovine serum albumin, Fucogel 1000PP) by using a Spectrum 100 FTIR

equipped with an Attenuated Total Reflectance (ATR) module and a crystal diamond. Principal Component Analyses (PCA) were realized (XLStats) to characterize and classify the IR spectrum of the CACR fraction among IR spectra of standards.

Molecular weight determination

Analysis of the carbohydrate fractions was carried out by HPLC using a Hewlett Packard series 1100. The following conditions were used to determine the molecular weight M_x of the polysaccharide by differential refractometry: 20 μ L of an aqueous solution of the purified polysaccharides (10 to 50 g/L) were injected into a TSK Gel G3000 PWXL-CP column at 40°C, using water as elution solvent at a flow rate of 0.7 mL/min. Dextran, dextran sulfate, blue dextran, heparin, fucoidan, glucose, lactose, raffinose were used as standards.

Carbohydrates monomers characterization

Acidic hydrolysis conditions, i.e. 4h at 90°C in 2M HCl, were performed on purified fractions to obtain samples containing mostly carbohydrates monomers. Preparations were then freeze-dried and stored at 20°C. Prior to carbohydrates characterization by HPLC (Hewlett Packard series 1100), 20 μ L of an aqueous solution of the fractions rich in monomers were injected into a ICSep ORH-801 at room temperature, using an aqueous solution of H₂SO₄ 0.01M as elution solvent at a flow rate of 0.6 mL/min. Analysis of the hydrolyzed carbohydrate fractions was also carried out by GC/MS using a Varian CP-3800 GC/Varian Saturn 2000. 400 mL of pyridine and 400 mL of BSTFA: TMCS (99:1) was added to 2 mg of purified polysaccharides. The solution was mixed for 2 h at room temperature, then injected into a DB-1701 J&W Scientific column (30 m, 0.32 mm, 1 mm) at a flow of 1 mL/min. The helium pressure was 8.8 psi. The temperature of the injector was set at 250°C. The rise in temperature in the oven was programmed for a first step at 150°C for 0 min, then an increment of 10°C/min up to 200°C with a final step at 200°C for 35 min. The ionization was performed by

119 Electronic Impact (EI, 70 eV), the trap temperature was set at 150°C and the target ion was
120 fixed at 40–650 m/z.

121 **Microbiological material and microorganism sources**

122 Growth media were from Biokar Diagnostics and antibiotic solutions (ampicillin and
123 cycloheximid) were from Sigma-Aldrich. The microorganisms used in this study, i.e.
124 *Staphylococcus aureus* (ATCC 25923), *Salmonella enteritidis* (ATCC 13076), *Pseudomonas*
125 *aeruginosa* (ATCC 27853), *Enterococcus faecalis* (CIP 103214), *Bacillus subtilis* (CIP 5262),
126 *Micrococcus luteus* (ATCC 4698) and *Candida glabrata* (DSMZ 6425), were obtained from
127 American Type Culture Collection (ATCC), Collection of Institute Pasteur (CIP) and German
128 Collection of Microorganisms and Cell Cultures (DSMZ). All of the microorganisms were
129 provided in the form of pure bacterial stock culture.

130 **Antibiotic susceptibility testing**

131 Antimicrobial susceptibility testing was determined by the disc diffusion method (Sanofi,
132 Dianostics Pasteur). However, the method was adapted for the use of microwells, instead of
133 employing discs, which allowed working with larger volumes and concentrations. The
134 photometric calibration method was used to adjust the inoculum of the microbial suspensions:
135 microbial suspensions were prepared in a phosphate buffer (0.1M pH 7.2) adjusting the cell
136 density to $1-3 \cdot 10^8$ microbial cells/mL, corresponding to an optical density between 0.10 and
137 0.12 at 550nm. Microbial suspensions were then diluted in Mueller Hinton media (MH) to 1:
138 100. MH or Yeast Peptone Dextrose (YPD) agar plates (for *Candida glabrata*) were flooded
139 by these suspensions (2mL). After 15min at 37°C, sterile microwells (in glass, internal
140 diameter: 4mm, external diameter: 6mm, height: 7mm) were put onto the microbial field and
141 inoculated by 30µL of the CACR fraction. It is noteworthy that these samples were previously
142 re-solubilized at 20 and 50mg.mL⁻¹ in different solvents: methanol, acetone,

dimethylsulfoxide (DMSO) and ethanol. Ampicillin and cycloheximid (10mg/ml) were used as positive controls. The plates were incubated at 30°C (for *Candida glabrata*) or 37°C and the zones of inhibition were observed after 24h.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) tests

The sensitivity of microorganism to CACR fractions was measured by using a tube dilution technique, which allows the determination of the MIC and MBC of the seaweed used in this study. These tests were done to determine the lowest concentration of the different extracts, where the bactericidal and bacteriostatic effect can be shown. The test was performed in tubes and allowed replicating each sample (triplicate). Microbial suspensions were prepared in the same way than previously. Microbial suspensions, diluted in MH at 1: 100, were added into increasing concentrations of CACR fractions, i.e. 2 to 50mg.mL⁻¹. The tubes were incubated at 30°C or 37°C during 24h. The first clear tube before turbid samples allowed the determination of the MIC. All clear tubes were placed out onto MH agar plates, and plates were incubated at 30°C or 37°C for 24h. Finally, the number of microorganism colonies developed on each agar plates was counted to determine the MBC, i.e. the plate where the concentration of the CACR fraction was sufficient to destroy 99.99% of the microorganism population. Ampicillin (10mg/ml) was used as positive control.

RESULTS AND DISCUSSION

Biochemical characterization

Many studies have highlighted that molecules from algae showed original biochemical compositions, behind nutritional [24], medical and antibacterial properties [25]. Seaweeds contain various compounds as polysaccharides, proteins, lipids, amino-acids, sterols or phenolic molecules which show bioactivity against microorganisms [26, 27] or virus [28]. On

the other hand, sulfated polysaccharides extracted from marine algae can be used for their anticoagulant and antithrombotic properties [29]. The main goal of this study was in a first time to find a valorization path of the macroalga *Chaetomorpha aerea*, which is an ecological problem for French west coast and especially oyster ponds. The extracellular polysaccharides of this green alga were firstly extracted and their compositions were characterized. Indeed, certain authors have highlighted that green algae as *Chaetomorpha* were composed of interesting polysaccharides (arabinogalactans), sometimes sulfated [16, 18]. The biochemical compositions (% w/w) of the control and the CACR fractions were determined (Table 1). The control fraction (CF) was composed of carbohydrates (3.82%), proteins (2.30%) and other compounds (93.9%) which were probably lipids, pigments and impurities. Owing to the extraction procedure, the CACR fraction obtained was composed of 76.6% carbohydrates, 17.3% proteins and the unknown part was reduced to 5.8%. From 5g of *Chaetomorpha aerea*, the method allowed the extraction of 233mg of the CACR fraction (extraction yield of 4.67%), whether approximately 178mg of carbohydrates and 40mg of proteins. The fraction was rich in carbohydrates and proteins (17.3%) (Table 1), which was coherent with previous works on a similar alga, *Chaetomorpha linum* [16]. It was noteworthy that the CACR fraction contained a large part of sulfated carbohydrates (6.3%) (Table 1). Besides, carbohydrates were sulfated (6.3%). In this way, Percival (1979) showed the important sulfatation degree of polysaccharides extracted from *Chaetomorpha* and a sulfated polysaccharide (11.9%) has been already purified from *Chaetomorpha antennina* [18].

FTIR analyses coupled to PCA allowed classifying the IR profile of the CACR fraction with various IR profiles of commercial polymers (Fig. 3). The general composition of this fraction was close to the composition of neutral and sulfated polysaccharides, as the dextran sulfate (17% sulfur). Owing to its composition, the dextran sulfate is known for its anticoagulant properties or its inhibitory effects against enzymes, cells or virus [30]. The general

composition and the sulfatation degree of the CACR fraction suggest that this extract could present similar biological activities.

Molecular weight determination and carbohydrates monomers characterization

Gel permeation chromatography analyses allowed the determination of the molecular weight of the CACR fraction. One peak was visible, at a retention time of 7.3min, by using the TSK Gel G3000 PWXL-CP column (Fig. 4, A). Comparing this retention time to the logarithmic standard curve, we concluded that the CACR fraction was composed of a main polysaccharide of $1.160 \times 10^6 \pm 0.150 \times 10^6$ Da. Other HPLC analyses, realized by using the ICSep ORH-801 fraction, showed that the CACR fraction was composed of three main monosaccharides: glucose, galactose and arabinose (Fig. 4, B). CPG/MS analyses confirmed the presence of glucose (24%), galactose (58%), arabinose (18%) and the traces of xylose (Table 2). The homology of MS spectra was verified (>91%), by comparing the MS spectra of standards and the MS spectra of the monosaccharides identified in the CACR fraction. According to previous studies [16, 18], the identification of a xyloarabinogalactan from the extracellular polysaccharides of a green alga was coherent and interesting. Xyloarabinogalactans, water-solubles and extracted from *Chlorophyceae*, are branched and sulfated heteropolysaccharides, presenting various composition, without repeating unit, except residues of (1,4)-L-arabinose separated by D-galactose units [16]. Although it is difficult to characterize the complete structure of this type of sulfated heteroglycans, numerous studies have highlighted the anti-herpetic, anti-coagulant activities or antioxidant capacity of sulfated galactans extracted from seaweeds [31].

Antibiotic susceptibility testing

The second part of this work was dedicated to a preliminary screening of the potential antibacterial activities of these natural extracted polysaccharides against various strains of

microorganisms, including pathogenic or resistant bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*), which contaminate many biological or inert surfaces. Inhibition zones were measured for each re-suspended CACR fractions (50mg.mL⁻¹). Positive controls confirmed that the strain correctly grew up and was affected by ampicillin. Negative controls showed that the solvents used did not affect the growth of the different microorganisms. First, no inhibition area was observed for the strains *Salmonella enteritidis* (ATCC 13076), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (CIP 103214) or *Candida glabrata* (DSMZ 6425) (Table 3). Only the strains *Bacillus subtilis* (CIP 5262), *Micrococcus luteus* (ATCC 4698) and *Staphylococcus aureus* (ATCC 25923) were affected by the presence of the CACR fraction resuspended in water (Table 3). Moreover, the strain of *Staphylococcus aureus* (ATCC 25923) was affected by different CACR fractions, re-suspended in different solvents (Fig. 5). *Staphylococcus aureus* (ATCC 25923) showed an important sensibility to the CACR fractions during the contact periods (Fig. 5). The methanol CACR extract showed the greatest diameter of inhibition, i.e. 13mm ± 1 (Fig. 5, D; Table 4), probably due to a better solubility of the molecules composing the CACR fraction (Table 4). It is important to note that the CACR fractions had an inhibitory activity only against three gram-positive microorganisms. Antimicrobial activities from seaweeds are mostly higher recurrent against gram positive bacteria as *Staphylococcus aureus* [32]. However, no inhibitory activity of the CACR fraction was found against *Enterococcus faecalis* (CIP 103214). Several authors clarified there is several reasons to explain why biological extracts could be active or not against different microbial strains, e.g. (i) the absence of target structure in the bacteria, (ii) the cell wall of the bacteria or (iii) the ability of the bacteria to modify the structure of the molecules composing the tested fraction [33].

MIC and MBC tests for the pathogenic strain *Staphylococcus aureus* (ATCC 25923)

Bacterial turbidities of *Staphylococcus aureus* (ATCC 25923) in contact with increasing concentrations of the CACR fraction (re-suspended in water or methanol) allowed the determination of the MIC and MBC. No bacterial turbidity, corresponding to the MIC values, was found at 40 and 42 mg.mL⁻¹ for the methanol and water CACR fractions respectively (Table 4). This result indicated that the CACR fraction could block the cell wall formation of this bacterium, inducing its lysis and death. However, it is noteworthy that the concentration of the antibacterial agent can greatly influence its classification as bacteriostatic or bactericidal agent. The protocol used to determine the MBC of the methanol and water CACR fractions highlighted a MBC value of 45 mg.mL⁻¹ for both. The MBC/MIC ratio indicated that the two CACR fractions presented a bactericidal activity against *Staphylococcus aureus* (ATCC 25923). However, a high dose of a bacteriostatic antibacterial molecule (as 45 mg.mL⁻¹) will be bactericidal [12]. Finally, several significant findings were found whereby the CACR fraction, composed of a sulfated xyloarabinogalactan, which exhibited a selective inhibitory activity against the Gram-positive bacterium *Staphylococcus aureus* (ATCC 25923). *Staphylococcus aureus* is a common pathogen spread by ingestion of contaminated food or water. Seafood is one of the sources of staphylococcal infection for humans [34]. Moreover, marine animals, as oysters, may be reservoirs or carriers of infectious agents and biological toxins [35]. In this way, studies have highlighted that oysters could be contaminated by *Staphylococcus aureus*, indirectly from contaminated water [36, 37]. The use of *Chaetomorpha aerea* in oyster ponds could be allowed to biologically purify water. The “secretion” of its extracellular sulfated galactan could avoid the microbial contamination of oysters against this pathogen bacterium, and finally could minimize the number of human food poisoning.

CONCLUSION

Therefore, this study allowed the extraction and partially characterization of an extracellular polysaccharide from the green alga *Chaetomorpha aerea*. The CACR fraction was mainly composed of one type of polysaccharide of 1.160×10^6 Da. This fraction contained 76.6% carbohydrates and 6.3% of them were sulfated. Chromatographic and GC/MS analyses highlighted that the polysaccharide is a xyloarabinogalactan, composed of 17.9% arabinose, 23.8% glucose, 58.3% galactose and some traces of xylose. Microbiological tests showed that the CACR fraction selectively inhibited the growth of *Staphylococcus aureus* (ATCC 25923) and could be potentially a bactericidal agent ($40 \text{ mg} \cdot \text{mL}^{-1}$). It could be interesting to better purify the CACR fraction by using ion exchange resins to eliminate the presence of proteins. On the other hand, the fact that the CACR fraction was composed of a sulfated xyloarabinogalactan is of primary interest since these kinds of algal sulfated heteroglycans are known for their biological activities as antimicrobial and especially anti-coagulant properties. The presence of this kind of polysaccharide could be a potential path to valorize the deleterious alga *Chaetomorpha aerea*.

Acknowledgements This study was financially supported by the Conseil Général of Charente-Maritime and the Centre National de la Recherche Scientifique.

REFERENCES

- [1] Zemke-White, W. L., and M. Ohno (1999) World seaweed utilization: an end-of-century summary. *J. Appl. Phycol.* 11: 369-376.
- [2] MacArtain, P., C. I. R. Gill, M. Brooks, R. Campbell, and I. R. Rowland (2007) Nutritional value of edible seaweeds. *Nutr. Rev.* 65: 535-543.
- [3] Pérez, R. (1997) Ces algues qui nous entourent. pp. 65-178. In: S. Arbault, O. Barbaroux, P. Phliponeau, C. Rouxel (eds.). *Aquaculture*. Editions IFREMER, Plouzané, France.

- 287 [4] Madhusudan, C., S. Manoj, K. Rahul, and C. M. Rishi (2011) Seaweeds: a diet with
288 nutritional, medicinal and industrial value. *Res. J. Med. Plant.* 5: 153-157.
- 289 [5] Val, A. G. D., G. Platas, A. Basilio, A. Cabello, J. Gorrochategui, I. Suay, F. Vicente, E.
290 Portillo, M. J. D. Río, G. G. Reina, and F. Peláez (2001) Screening of antimicrobial activities
291 in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int.*
- 292 [6] Nakajima, K., A. Yokoyama, and Y. Nakajima (2009) Anticancer effects of a tertiary
293 sulfonium compound, dimethylsulfoniopropionate, in green sea algae on Ehrlich ascites
294 carcinoma-bearing mice. *J. Nutr. Sci. Vitaminol.* 55: 434-438.
- 295 [7] Boisson-Vidal, C., F. Zemani, G. Calliguirri, I. Galy-Fauroux, S. Collic-Jouault, D.
296 Helley, and A. M. Fisher (2007) Neoangiogenesis induced by progenitor endothelial cells:
297 effect of fucoidan from marine algae. *Cardiovasc. Hematol. Agents Med. Chem.* 5: 67-77.
- 298 [8] Synytsya, A., W. -J. Kim, S. -M. Kim, R. Pohl, A. Synytsya, F. Kvasnička, J. Čopíková,
299 and Y. Il Park (2010) Structure and antitumour activity of fucoidan isolated from sporophyll
300 of Korean brown seaweed *Undaria pinnatifida*. *Carbohydr. Polym.* 81: 41-48.
- 301 [9] Tringali, C. (1997) Bioactive metabolites from marine algae: recent results. *Curr. Org.*
302 *Chem.* 1: 375-394.
- 303 [10] Selvin, J., and A. P. Lipton (2004) Biopotentials of *Ulva fasciata* and *Hypnea*
304 *musciformis* collected from the Peninsular Coast of India. *J. Mar. Sci. Technol.* 12: 1-6.
- 305 [11] European Antimicrobial Resistance Surveillance System (EARSS) (2004) *Annual Report*
306 *EARSS-2003*. Pp. 90-91. NBilthoven, The Netherland.

- 307 [12] Al-Haj, N. A., N. I. Mashan, M. N. Shamsudin, H. Mohamad, C. S. Vairappan, and Z.
308 Sekawi (2009) Antibacterial activity in marine algae *Eucheuma denticulatum* against
309 *Staphylococcus aureus* and *Streptococcus pyogenes*. *Res. J. Biol. Sci.* 4: 519-524.
- 310 [13] Kim, I. H., S. H. Lee, J. -M. Ha, B. -J. Ha, S. -K. Kim, and J. -H. Lee (2007)
311 Antibacterial activity of *Ulva lactuta* against Methicillin-Resistant *Staphylococcus aureus*
312 (MRSA). *Biotechnol. Bioprocess Eng.* 12: 579-582.
- 313 [14] Chebil Ajjabia, L., and L. Choubab (2009). Biosorption of Cu^{2+} and Zn^{2+} from aqueous
314 solutions by dried marine green macroalga *Chaetomorpha linum*. *J. Environ. Manage.* 90:
315 3485-3489.
- 316 [15] Anand Ganesh, E., S. Das, G. Arun, S. Balamurugan, R. Ruban Raj (2009) Heparin like
317 compound from green alga *Chaetomorpha antennina* - as potential anticoagulant agent. *Asian*
318 *J. Med. Sci.* 1: 114-116.
- 319 [16] Percival, E. (1979) The polysaccharides of green, red and brown seaweeds: their basic
320 structure, biosynthesis and function. *Br. Phycol. J.* 14: 103-117.
- 321 [17] Genestie, B. (2006) *Optimisation de la production d'arabinoxyloligosaccharides*
322 *d'intérêt biologique à partir de sons de céréales : approches méthodologiques*. Ph.D. Thesis.
323 University of Limoges, Limoges, France.
- 324 [18] Venkata Rao, E., and K. Sri Ramana (1991) Structural studies of a polysaccharide
325 isolated from the green seaweed *Chaetomorpha antennina*. *Carbohydr. Res.* 217: 163-170.
- 326 [19] Guetta, O., K. Mazeau, R. Auzely, M. Milas, and M. Rinaudo (2003) Structure and
327 properties of a bacterial polysaccharide named Fucogel. *Biomacromol.* 4: 1362-1371.

- 328 [20] Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith (1956) Colorimetric
329 method for determination of sugars and related substances. *Anal. Chem.* 28, 350-356.
- 330 [21] Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D.
331 Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk (1985) Measurement
332 of protein using bicinchoninic acid. *Anal. Biochem.* 150: 76-85.
- 333 [22] Jaques, L. B., R. E. Ballieux, C. P. Dietrich, and L. W. Kavanagh (1968) A
334 microelectrophoresis method for heparin. *Can. J. Physiol. Pharmacol.* 46: 351-360.
- 335 [23] Craigie, J. S., Z. C. Wen, and J. P. van der Meer (1984) Interspecific, intraspecific and
336 nutritionally-determined variations in the composition of agars from *Gracilaria spp.* *Bot.*
337 *Mar.* 27: 55-61.
- 338 [24] Fleurence, J. (1999) Seaweed proteins: biochemical, nutritional aspects and potential
339 uses. *Trends Food Sci. Technol.* 10: 25-28.
- 340 [25] Chakraborty, K., A. P. Lipton, R. Paul Raj, K. K. Vijayan (2010) Antibacterial labdane
341 diterpénoides of *Ulva fasciata Delile* from southwestern coast of the India Peninsula. *Food*
342 *Chem.* 119:1399-1408.
- 343 [26] Wong, W. H., S. H. Goh and S. M. Phang (1994) Antibacterial properties of Malaysian
344 seaweeds. pp. 75-81. In: S. M. Phang, Y. K. Lee, M. A. Borowitzka, and B. A. Whitton (eds.).
345 Algal biotechnology in the Asia-Pacific region. University of Malaya, Kuala Lumpur,
346 Malaysia.
- 347 [27] Nirmal Kumar, J. I., R. N. Kumar, M. K. Amb, A. Bora, and S. Kraborty (2010)
348 Variation of biochemical composition of eighteen marine macroalgae collected from Okha
349 Coast, Gulf of Kutch, India. *Electron. J. Environ. Agric. Food Chem.* 9: 404-410.

- 350 [28] Abrantes, J. L., J. Barbosa, D. Cavalcanti, R. C. Pereira, C. L. Frederico Fontes, V. L.
351 Teixeira, T. L. Moreno Souza, and I. C. P. Paixão (2010) The effects of the diterpenes isolated
352 from the Brazilian brown algae *Dictyota pfaflii* and *Dictyota menstrualis* against the herpes
353 simplex type-1 replicative cycle. *Planta Med.* 76: 339-344.
- 354 [29] Mao, W., X. Zang, Y. Li, and H. Zhang (2005) Sulfated polysaccharides from marine
355 green algae *Ulva conglobata* and their anticoagulant activity. *J. Appl. Phycol.* 18 :9-14.
- 356 [30] Baba, M., R. Snoeck, R. Pauwels, and E. de Clerq (1988) Sulfated polysaccharides are
357 potent and selective inhibitors of various enveloped viruses, including herpes simplex virus,
358 cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrob*
359 *Agents Chemother.* 32: 1742-1745.
- 360 [31] Barahona, T., N. P. Chandía, M. V. Encinas, B. Matsuhira, and E. A. Zúñiga (2011)
361 Antioxidant capacity of sulfated polysaccharides from seaweeds. A kinetic approach. *Food*
362 *Hydrocolloids.* 25: 529-535.
- 363 [32] Reichelt, J. L., and M. A. Borowitzka (1984) Antimicrobial activity from marine algae:
364 results of a large scale screening program. *Hydrobiologia.* 116-117: 158-168.
- 365 [33] Michael, T. M., M. M. John, and P. Jack (2002) Brock microbiology of microorganism.
366 10th ed. Prentice Hall, New Jersey, US.
- 367 [34] Liston, J. (1990) Microbial hazards of seafood consumption toxins, bacteria and viruses
368 are the principal causes of sea foodborne diseases. *Food Technol.* 44: 58-62.
- 369 [35] Jeyasekaran, G., I. Karunasagar, and I. Karunasagar (1996) Incidence of *Listeria spp.* in
370 tropical fish. *Int. J. Food Microbiol.* 1-3 : 333-340.

371 [36] Beleneva, I. A. (2010) Incidence and characteristics of *Staphylococcus aureus* and
372 *Listeria monocytogenes* from the Japan and South China seas. Mar. Pollut. Bull. doi:
373 10.1016/j.marpolbul.2010.09.024.

374 [37] Oliveira, J., A. Cunha, F. Castilho, J. L. Romalde, and M. J. Pereira (2011) Microbial
375 contamination and purification of bivalve shellfish: crucial aspects in monitoring and future
376 perspectives – a mini-review. Food Control. 22: 805-816.

377

378 Table 1. Biochemical composition (% w/w) of the native sample and the CACR fraction
 379 extracted from *Chaetomorpha aerea*.

Composition (% w/w)	Carbohydrates	Uronic acids	Proteins	Sulfated carbohydrates	Others
Control fraction	3.82 ± 0.12	nd	2.30 ± 0.11	0.33 ± 0.14	93.9
Partially purified fraction	28.1 ± 2.31	nd	6.53 ± 1.08	2.21 ± 0.92	71.9
CACR fraction	76.6 ± 0.62	nd	17.3 ± 2.85	6.3 ± 1.2	5.8

380 *nd: non determined by colorimetric assays*

381 \pm : Standard deviation σ_x on 10 runs.

382

383 Table 2. Identification and quantification by GC/MS of the monosaccharides composing the
 384 CACR fraction extracted from *Chaetomorpha aerea*.

Monosaccharides	Retention time of standards	Retention time of peaks in the CA fraction	Homology MS spectra (%)	Concentration (% w/w)
Rhamnose	6.49	-	-	-
Arabinose	6.61	6.59	93.45	18
Fucose	7.65	-	-	-
Xylose	8.59	9.11	91.87	trace
Mannose	10.72	-	-	-
Galactose	11.73	11.72	98.12	58
Glucose	12.67	12.59	98.89	24
Inositol	23.89	-	-	-

385

386

387 Table 3. Antibacterial activities against the different gram-positive/gram-negative bacteria of
 388 the CACR extract, previously re-solubilized in water.

Microorganisms	Diameters of inhibition (mm)
<i>Bacillus subtilis</i> (CIP 5262)	14 ± 2
<i>Micrococcus luteus</i> (ATCC 4698)	13 ± 2
<i>Staphylococcus aureus</i> (ATCC 25923)	11 ± 0.5
<i>Enterococcus faecalis</i> (CIP 103214)	0
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	0
<i>Salmonella enteritidis</i> (ATCC 13076)	0
<i>Candida glabrata</i> (DSMZ 6425)	0
Ampicillin (positive control)	41 ± 1.5

389 ±: Standard deviation σ_x on 10 runs.

390

391

Table 4. Antibacterial activities of the CACR extract against *Staphylococcus aureus* (ATCC 25923), previously re-solubilized in different solvents.

Solvents used to solubilize extracts	<i>Diameters of inhibition (mm)</i>
Water (50mg.mL⁻¹)	11 ± 0.5
Ethanol (50mg.mL⁻¹)	11 ± 1
Acetone (50mg.mL⁻¹)	12 ± 0.5
Methanol (50mg.mL⁻¹)	13 ± 1
Dimethylsulfoxyde (50mg.mL⁻¹)	10 ± 0.5
Ampicillin (10mg.mL⁻¹)	41 ± 1.5

±: Standard deviation σ_x on 10 runs.

397 Table 5. Bacterial turbidity of *Staphylococcus aureus* (ATCC 25923) after 24h at 37°C in
 398 contact with different concentrations of the CACR extract, previously re-suspended in water
 399 or methanol. Ampicillin was used as positive control.

Concentrations (mg.mL ⁻¹)	Ampicillin	CACR water-fractions	CACR methanol-fractions
2	-	+++	+++
4	-	+++	+++
8	-	+++	+++
10	-	+++	+++
15	-	+++	+++
20	-	+++	+++
30	-	++	++
35	-	+	+
40	-	+	-
42	-	-	-
45	-	-	-
50	-	-	-

+++ : very turbid microbial suspension

++: turbid microbial suspension

+: low turbid microbial suspension

-: no turbidity

400

401

402 Figure 1. *Chaetomorpha aerea*, the type of green alga which was used in this study.

403 Figure 2. Extraction and purification procedure which was developed to obtain the CACR
404 fraction from the mucilage of *Chaetomorpha aerea*.

405 Figure 3. Principal Component Analysis (PCA) (XLStat) of the FTIR spectra obtained for the
406 CACR fraction and different commercial polysaccharides and proteins. The spectral region
407 selected for the PCA analysis was comprised between 650 and 4000cm⁻¹.

408 Figure 4. Analyses by HPLC: determination of the molecular weight by gel permeation
409 chromatography (A) and characterization of the monosaccharides distribution (B) of the
410 polymer composing the CACR fraction.

411 Figure 5. Sensibility of *Staphylococcus aureus* (ATCC 25923), after 24h at 37°C, against
412 different re-suspended CACR fraction (50mg.mL⁻¹): water (A), ethanol (B), acetone (C) and
413 methanol (D) CACR fractions. T+ corresponded to the sensibility of the strain against
414 ampicillin. T- showed the non-effect of the solvent used on the strain growth.

415

416

417

418



419

420

421

FIGURE 1

422

423

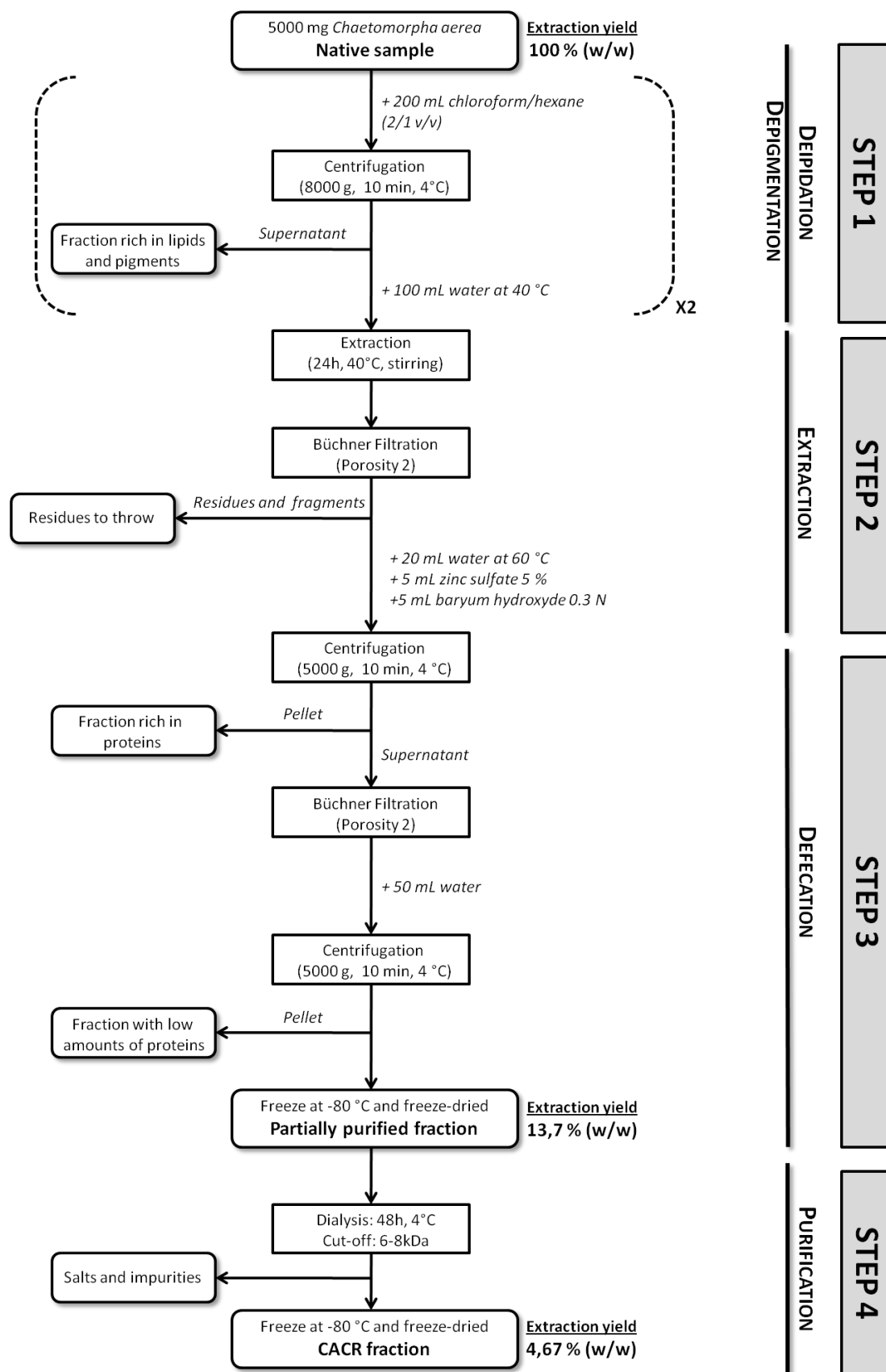


FIGURE 2

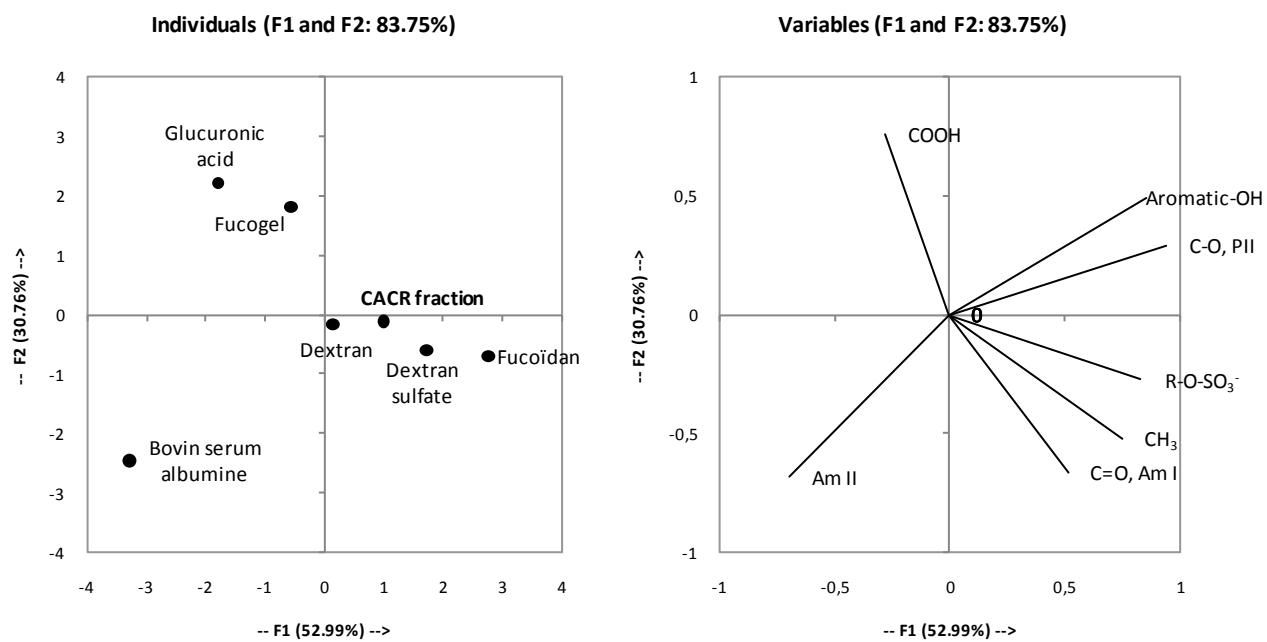


FIGURE 3

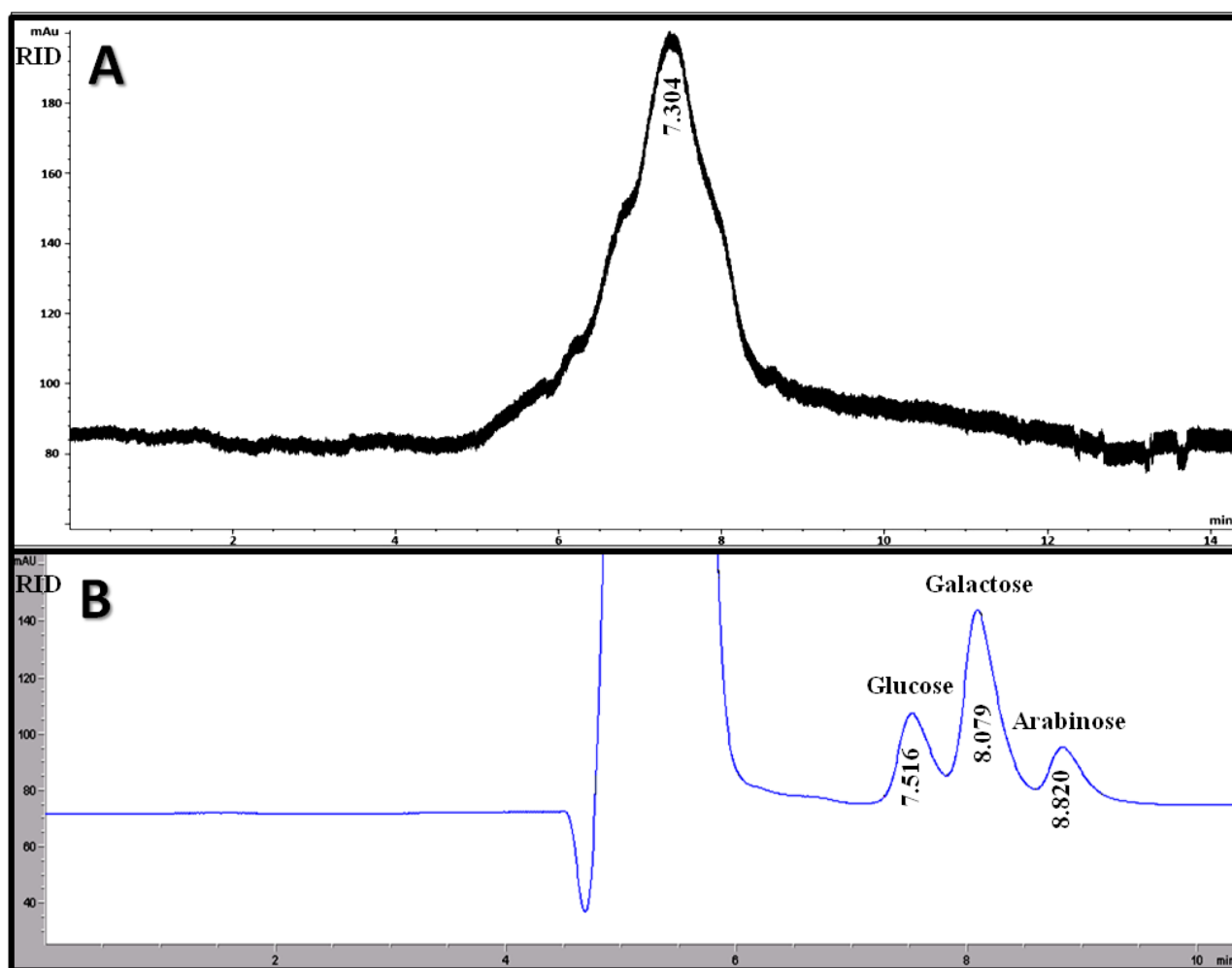
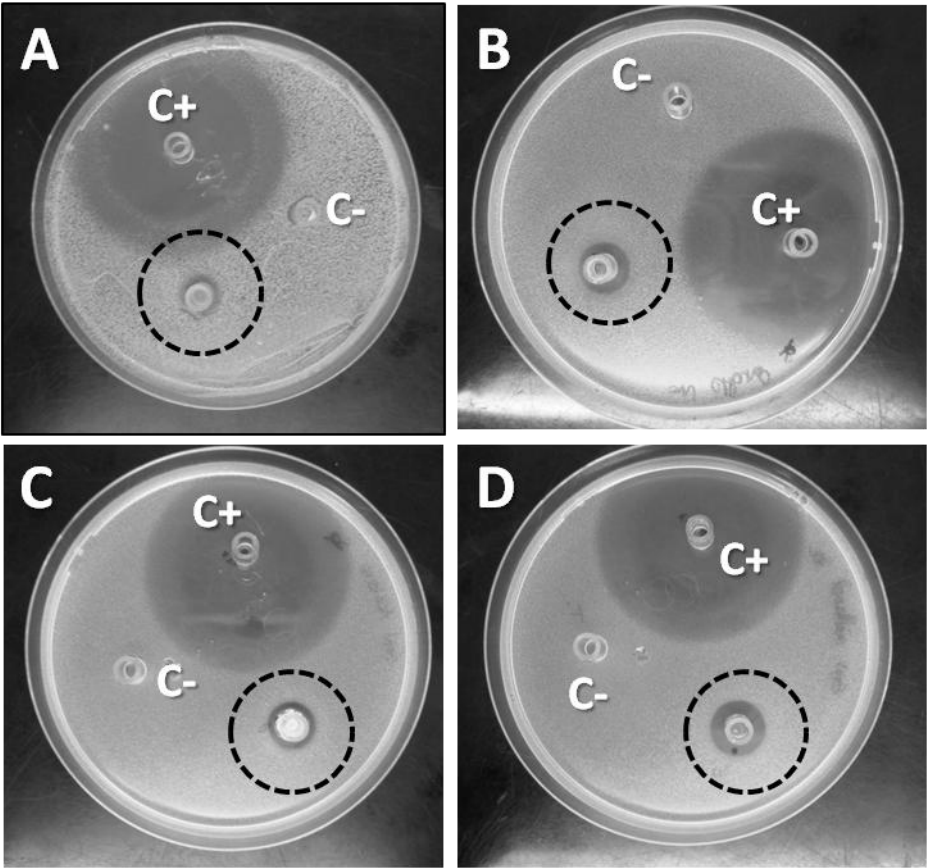


FIGURE 4

437



438

439

440

441

442

FIGURE 5