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Antibacterial activity of a new lectin isolated from the marine sponge *Chondrilla caribensis*

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ABSTRACT

A new lectin from the marine sponge *Chondrilla caribensis* (CCL) was isolated by affinity chromatography in Sepharose 6B media. CCL is a homotetrameric protein formed by subunits of 15,445 ±2 Da. The lectin showed affinity for disaccharides containing galactose and mucin. Mass spectrometric analysis revealed about 50% of amino acid sequence of CCL, which showed similarity with a lectin isolated from *Aplysina lactuca*. Secondary structure consisted of 10% α-helix, 74% β-sheet/β-turn and 16% coil, and this profile was unaltered in a broad range of pH and temperatures. CCL agglutinated *Staphylococcus aureus*, *S epidermidis* and *Escherichia coli*, and it was able to reduce biofilm biomass, but showed no inhibition of planktonic growth of these bacteria. CCL activity was inhibited by α-lactose, indicating that Carbohydrate Recognition Domain (CRD) of the lectin was involved in antibiofilm activity.

1. Introduction

Lectins represent a class of proteins with ability to bind to specific sugars in a non-catalytic way. The lectins are ubiquitous proteins, being found in all living organisms. In Porifera phylum, approximately fifty lectins were isolated and biochemically characterized [1].

Interestingly, lectins from marine sponge showed a broad spectrum of biological activities [1]. For instance CaL, a lectin isolated from *Cinachyrella apion*, showed high growth inhibition for HeLa, reducing cell growth at a dose dependent manner [2]. Other lectin, CvL, isolated from *Cliona varians*, inhibited the growth of human leukemia cells, but no effect on heath blood lymphocytes was observed [3].

Moreover, lectins from marine sponges are involved in self-defense of the organism, since several lectins are able to recognize, agglutinate and inhibit growth of bacterial cells and biofilms [1]. ALL, a lectin isolated from *Aplysina lactuca*, agglutinated Gram-positive and Gram-negative bacterial cells, and it were able to reduce the biomass of bacterial biofilms at dose-dependent effect [4]. On the other hand, a lectin from *Cliona varians* (CvL) displays a cytotoxic effect on Gram-positive bacteria, but did not affect Gram-negative bacteria [5].

In addition to marine sponges, others invertebrate lectins have showed interesting biological properties [6,7]. MytiLec isolated from *Mytilus galloprovincialis* showed a dose-dependent cytotoxic effect on human Burkitt lymphoma Raji cells [8], whereas ADEL

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from *Aplysia dactylomela* eggs was able to agglutinate and inhibit biofilm formation of *Staphylococcus aureus* [9].

Chondrilla caribensis Ruetzler, Duran & Piantoni (2007) is a grayish to chestnut and purplish brown sponge commonly found in marine reefs, where it inhabits solid substrate such as rock, conch shells and red-mangrove stilt roots. In this work, we reported the purification and biochemical characterization of new lectin from *C. caribensis* with antibiofilm activity.

2. Material and methods

2.1. Animal collection

C. caribensis specimens were collected in the intertidal zone in the Paracuru beach, Ceará, Brazil. The animals were transported in plastic bags to the lab and stored at −20 °C until use. The species was identified, and a voucher was deposited (ID: UFPEPOR2254) at the Zoology Department of the Universidade Federal de Pernambuco, PE, Brazil. Collections were authorized and certified by responsible environmental institutions (SISBIO ID: 33913-8).

2.2. CCL purification

Frozen sponges were freeze-dried and triturated to a fine powder. Sponge powder was soaked with ten volumes (w:v) of 50 mM Tris-HCl, pH 7.6, containing NaCl 150 mM and PMSF 0.1 mM (TBS), and maintained under agitation for 30 min at room temperature. Then, material was centrifuged at 8000×*g* for 20 min at 4 °C, and the supernatant, named crude extract, was stored at −20 °C until the use.

Crude extract was loaded into HCl-activated Sepaharose™ 6 B column (1.0 × 10.0 cm), previously equilibrated with TBS. Unbound proteins were washed with same buffer and the lectin was recovered through elution with 0.2 M lactose in TBS. Fractions eluted with lactose in TBS were pooled, dialyzed against distillated water, freeze-dried and stored until the use.

Chromatography was conducted at flow rate of 1 mL min^{-1}, and monitored by measurement of absorbance at 280 nm 2-mL fractions were collected.

2.3. Hemagglutinating activity and inhibition

Hemagglutinating activity and inhibition assays was carried according to pre-established methods [10]. Erythrocytes from human (A, B and O) and rabbit were used in their native and protease (trypsin and papain) treated forms.

In the inhibition assay, the following sugars and glycoproteins were used: D-xylose, D-ribose, L-fucose, L-arabinose, L-rhamnose, D-galactose, D-mannose, D-glucose, D-glucosamine, D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-galacturonic acid, D-fructose, D-sucrose, D-melibiose, α-D-lactose, β-D-lactose, D-lactulose, D-maltose, D-raffinose, methyl-α-D-galactopiranoside, methyl-β-D-galactopiranoside, methyl-β-D-thiogalactose, phenyl-β-D-galactopiranoside, 4-nitrophenyl-α-D-galactopiranoside, 4-nitrophenyl-β-D-galactopiranoside, 2-nitrophenyl-β-D-galactopiranoside, O-nitrophenyl-β-D-galactopiranoside, bovine submaxillary mucin (BSM), porcine fetuin and porcine stomach mucin (PSM) type 2 and 3.

The effect of the pH, temperature and divalent ions on hemagglutinating activity of CCL was evaluated as described by Sampaio, Rogers, & Barwell [10].

2.4. Molecular mass determination and sugar content

Molecular mass of CCL under denaturing conditions was estimated by SDS PAGE [11], in the presence and absence of β-mercaptoethanol. LMW-SDS marker kit (GE Healthcare, UK) was used as standard.

Native molecular mass of CCL was estimated by size exclusion chromatography (SEC) on BioSuite 250 HR SEC column (0.78 × 30 cm, 5 μm particle size, Waters Corp.) coupled to an HClass UPLC system (Waters Corp, MA, USA). 200 μg of CCL were applied into the column, previously equilibrated with TBS. The column was calibrated with BSA (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), trypsnogen (24 kDa), and ribonuclease A (13.7 kDa).

Molecular mass of CCL was determined by Electrospray ionization – Mass Spectrometry (ESI–MS), conform described by Carneiro et al. [4] using a hybrid Synapt HDMS mass spectrometer (Waters Corp., Milford, MA, USA). The instrument was calibrated with [Glu1]fibrinopeptideB fragments. Mass spectra were acquired by scanning at *m*/*z* range from 500 to 3000 at 1 scan/s.

Free and total cysteines in CCL were quantified conform described by Carneiro et al. [4].

Neutral carbohydrate content in CCL was evaluated, as described by Dubois et al. [12], using lactose as the standard.

2.5. Tandem mass spectrometry (MS/MS) of tryptic peptides

Digestion with trypsin and peptide extraction was carried out as described by Shevchenko et al. [13]. Tryptic peptides were separated on a reverse phase C-18 (0.075 × 100 mm) nanocolumn coupled to a nanoAcquity system. The eluates were analyzed in a hybrid mass spectrometer (ESI-Q-ToF) (Synapt HDMS, Waters Corp. MA, USA). The instrument parameters were adjusted as described by Carneiro et al., [14].

Collision induced dissociation (CID) spectra were manually interpreted, and sequenced peptides were searched online against NCBI and Uniprot protein databanks.

2.6. Circular dichroism analysis

Circular Dichroism (CD) spectroscopic measurement was performed on a Jasco J-815 spectropolarimeter (Jasco International Co., Tokyo, Japan) connected to a peltier with controlled temperature conform described by Carneiro et al., [9].

The effect of the pH on the CCL secondary structure was determined by incubation of the lectin in different pH values (2, 4, 7, 9 and 12) for 1 h followed by CD measurements. The effect of the temperature on the CCL secondary structure was determined by CD measurements in different temperatures (20, 37, 56, 72 and 95 °C). The effect of sugars in the CCL fold was observed by CD measurements in the near UV, conform described by Carneiro et al., [9].

2.7. Antibacterial activity

2.7.1. Strain and culture conditions

Three reference strains from the American Type Culture Collection (ATCC) were used in this study: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228 and *Escherichia coli* ATCC 11303.

Bacteria were grown in Trypticase Soy Agar medium (TSA; Himedia, India) and incubated at 37 °C for 24 h. After growth on TSA, isolated colonies were inoculated in 10 mL of Trypticase Soy Broth (TSB; Himedia, India) and incubated under constant agitation

at 37 °C for 24 h. Then, the bacterial suspensions were prepared in TSB adjusted at a cell density of 2×10^6 cfu/mL.

2.7.2. Bacterial agglutination

The bacteria were grown in TSB at 37 °C for 24 h and harvested by centrifugation at 2000×*g* for 10 min. Agglutination assays were performed as described by Melo et al. [15].

2.7.3. Effects of CCL on planktonic cells

The antibacterial activity of CCL was evaluated by the microdilution method according to the Clinical and Laboratory Standards Institute document M07-A9, with some modifications [16,17].

2.7.4. Effect of CCL on biofilm formation

The methodology used to evaluate the effects of the lectin on biofilm formation was based on the microtiter plate test described by Stepanovic et al. [18] with some modifications. The cellular suspensions adjusted 2×10^6 cells mL^{-1} were transferred to 96-well microtiter plates (100 μL per well) and then added CCL in different concentrations (7.8–250 μg mL^{-1}). To promote biofilm development, the plates were incubated for 24 h at 37 °C. The biofilm formation was evaluated by two distinct methods: biomass quantification by crystal violet staining and enumeration of biofilm-entrapped viable cells.

2.7.4.1. Quantification of the biofilm biomass. The biomass of the biofilms formed in presence and absence of the lectin was analyzed using the crystal violet staining method. For that, the wells containing the biofilms were washed twice with 200 μL with ultrapure water to remove weakly adherent cells and left to air dry. The biofilms were then fixed with 200 μL of 99% methanol for 15 min. After 15 min, the methanol was removed, the wells were dried at room temperature and then 200 μL of crystal violet stain (1%, v/v) were added to the wells and after 5 min, the excess of crystal violet was removed. After the staining step, the plates were washed and air dried for approximately 20 min. Lastly, 200 μL of acetic acid (33%, v/v) were added to each well to solubilize the bound crystal violet. The absorbance of each well was measured at 590 nm (OD_{590}) using a microplate reader (SpectraMax® I3).

2.7.4.2. Quantification of biofilm-entrapped cells. In order to determine viability of biofilm-entrapped cells treated and untreated with CCL, the wells were washed twice with 200 μL of ultrapure water and the biofilm suspensions removed by sonication for 10 min and then serially diluted and plated on TSA [16]. After incubation at 37 °C for 24 h, the total number of colony-forming units (CFUs) was quantified and expressed as CFU/mL.

2.7.5. Effect of lactose on antibiofilm activity of CCL

To verify the role of the carbohydrate recognition domain (CRD) on the antibiofilm activity of CCL, the lectin (250 μg mL^{-1}) was incubated with 100 mM α-lactose at 37 °C for 1 h. After incubation, the biofilm of *S. aureus* and *S. epidermidis* were developed in presence of CCL with or without α-lactose. The biofilm formation was evaluated by biomass quantification as described previously.

2.7.6. Statistical analysis

Statistical analyses were performed by GraphPad Prism® version 5.0 for Microsoft Windows®. Data from all assays were compared using one-way analysis of variance (ANOVA), with Bonferroni post hoc test. Data were considered significant when $P < 0.05$.

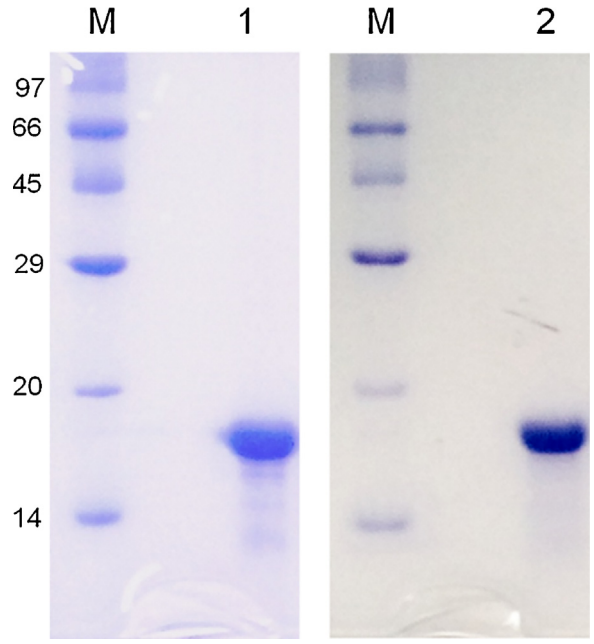

Fig. 1. SDS-PAGE of purified CCL. SDS-PAGE 15%. M) Molecular Marker. 20 μg of CCL were applied in the absence (1) and presence of β-mercaptoethanol (2).

3. Results

3.1. CCL purification

The crude extract of *C. caribensis* showed hemagglutinating and hemolytic activity against all tested erythrocytes. In affinity chromatography, CCL was adsorbed in the Sepharose matrix, and it was successfully recovered by elution with lactose (data not shown). Nevertheless, hemagglutinating activity was observed in non-retained fractions. CCL was purified 21 times and represented 34% of the total hemagglutinating activity of the extract (Table 1)

In SDS PAGE, under non-reducing and reducing conditions, CCL exhibited one single band with apparent molecular mass of 17 kDa (Fig. 1).

3.2. Hemagglutinating and inhibition

CCL agglutinated all tested erythrocytes with preference for rabbit erythrocytes. Moreover, enzyme treated-erythrocytes were better agglutinates than those non-treated (Table 2).

Among all tested sugars, only lactose, lactulose at concentration of 100 mM, fetuin, BSA, PSM type 2 and 3 were able to inhibit CCL hemagglutinating activity (Table 3).

Table 1
Purification of CCL.

Fraction	Volume (mL)	Titer (HU mL^{-1})	Protein total (mg)	Hemagglutinating activity total (HU)	Specific activity (HU mg^{-1})	Purification (fold)	Yield (%)
Crude extract	50	128	395	6400	16.2	1	100
CCL	17	128	6.3	2176	346	21	34

Table 2
Hemagglutinating activity of CCL against distinct erythrocytes.

Erythrocyte/treatment	Non-treated	Enzyme-treated	
		Trypsin	Papain
Human A	256	512	512
Human B	256	512	512
Human O	256	512	512
Rabbit	512	1024	1024

Suspension of erythrocytes were prepared at 3%; Hemagglutination was expressed in titer of hemagglutination.

Table 3
Inhibitory substance of CCL.

Sugar	MIC[a]
α-lactose (Galβ1 → 4α-Glc)	100 mM
β-lactose (Galβ1 → 4β-Glc)	100 mM
Lactulose (Galβ1 → 4Fru)	100 mM
Glycoproteins	
BSM	32 μg mL^{-1}
Fetuin	16 μg mL^{-1}
PSM type 2	8 μg mL^{-1}
PSM type 3	8 μg mL^{-1}

[a] Minimal Inhibition Concentration.

3.3. Effect of the pH, temperature and divalent cations on hemagglutinating activity

Maximum activity of CCL was observed at pH 9, below and above pH 9 hemagglutinating activity decreases, at pH 10 residual activity was observed, while at pH 4 there was complete loss of activity (data not shown).

CCL is a high thermostable protein. Only after boil for 60 min its activity was fully lost. EDTA, $CaCl_2$ and $MnCl_2$ did not affect CCL activity.

3.4. Molecular mass determination and sugar content

ESI–MS revealed molecular mass of 15,445 ±2 Da with slight differences around this value, indicating the presence of micro-heterogeneities in the amino acid sequence of CCL (Fig. 2).

No cysteine was found in CCL structure, since no mass shift were observed by ESI–MS after treatment with iodoacetamide (IAA) and dithiothreitol followed of IAA (data not shown).

In size exclusion chromatography, CCL was eluted as a sharp and symmetrical peak of 54 kDa, suggesting an oligomeric structure composed of four identical 15 kDa subunits linked by weak interactions (Fig. 3).

The phenol-sulfuric acid assay indicated that CCL is a glycoprotein with 5.2% of neutral carbohydrate.

3.5. MS/MS

Six peptides were sequenced by MS/MS (Table 4). Together, these peptides correspond to approximately 50% of the amino acid sequence of CCL. Peptides T1, T2 and T-4 showed slight similarity with peptides from ALL, a lectin isolated from marine sponge *Aplysina lactuca*. T1 and T2 showed 75% and 62% of identity with T6 from ALL, whereas T4 presented 50% of identity with ALL T5 (data not shown).

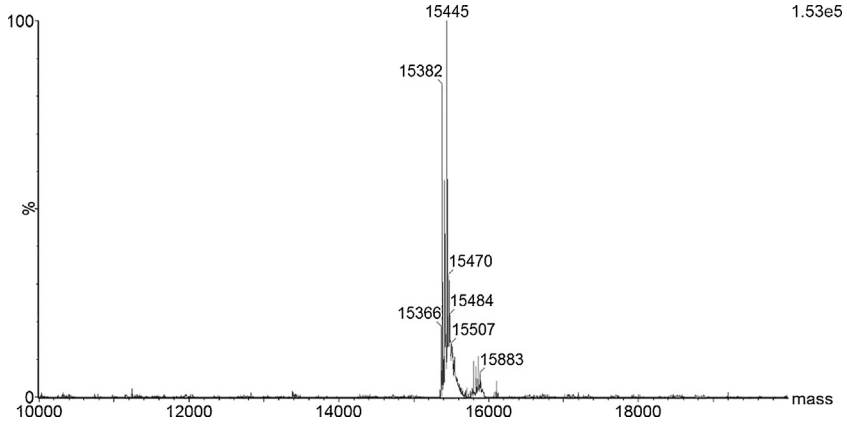

Fig. 2. Molecular mass determination of CCL by Electrospray ionization mass spectrometry (ESI–MS). A) Deconvoluted ESI–MS of CCL. The lectin (10 pmol μL-1) was dissolved in Acetonitrile 50% containing 0.2% formic acid and infused into the NanoESI source coupled to an ESI-Q-ToF mass spectrometer.

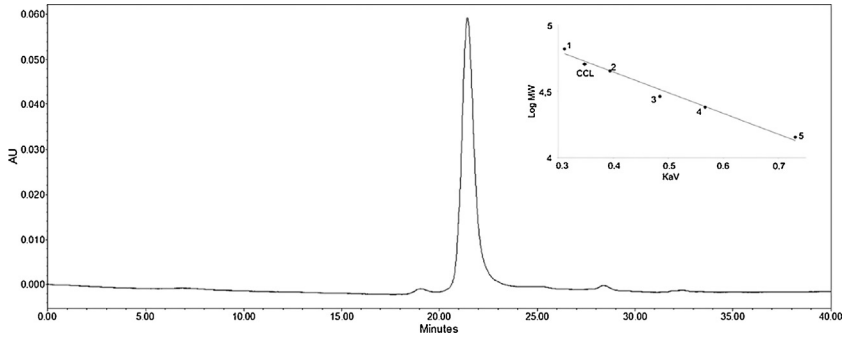

Fig. 3. Size exclusion chromatography of CCL. Approximately 200 μg of CCL were loaded into BioSuite 250 HR SEC column (0.78 × 30 cm, 5 μm particle size, Waters Corp.). The column was equilibrated and eluted with 50 mM Tris-HCl, pH 7.2, containing NaCl 150 mM. UPLC operated at flow of 0.5 mL min-1. Insert. Column calibration: 1. BSA (66 kDa); 2. Ovoalbumin (45 kDa); Carbonic anhydrase (29 kDa); 4. Trypsinogen (24 kDa); 5. Lisozyme (14,3 kDa).

Table 4
Peptides originated by digestion of CCL with trypsin.

Peptide	Amino acid Sequence	Mass		Δ (Da)
		Observed	Calculated	
T-1	[L/I]PVNSVQR	911.47	911.52	0.05
T-2	[L/I]PVNSVK	755.34	755.45	0.11
T-3	GVGQSATAVYTAPGDGR	1605.74	1605.77	0.03
T-4	S[L/I]DGHFA[L/I][L/I]ADG[L/I]QVATYDHR	2298.05	2298.13	0.13
T-5	VATVS[L/I]PR	841.46	841.50	0.04
T-6	TD[L/I]N[L/I][L/I]DADGGYV[L/I]HEDYR	2196.00	2196.04	0.04

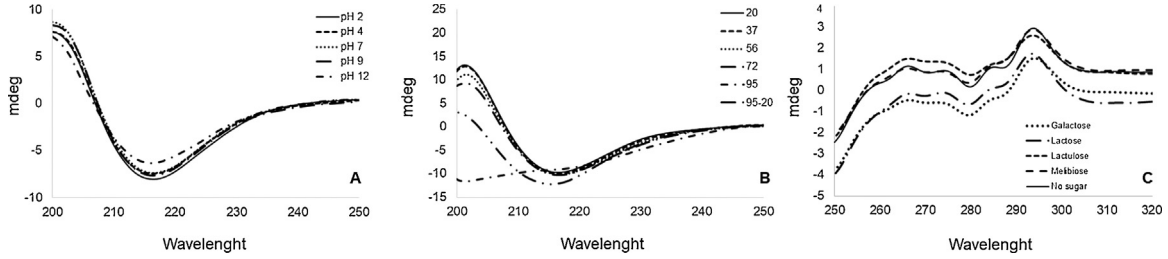

Fig. 4. CD spectra of CCL. (A) Far-UV CD spectra (200–250 nm) of CCL incubated at different pH. (B) Far-UV CD spectra (200–250 nm) of CCL incubated at different temperatures (°C). (C) Near-UV spectra (250–320 nm) of CCL in the presence of sugars.

3.6. Dichroism circular

CD spectra of native CCL exhibited one minimum at 217 nm, suggesting a predominance of β-conformation in its secondary structure. The prediction method CONTIN [19] indicated that the theoretical secondary structure consisted of 10% α-helix, 74% β-sheet/β-turn and 16% coil.

No significant modifications in the maximum and minimum absorption were observed when CCL was incubated in pH 2–10. The secondary structure of the lectin at pH 12 was slightly affected (Fig. 4A)

No significant changes in secondary structure were observed between 20 °C and 75 °C, but after heating at 95 °C deep modifications were detected in the maximum and minimal absorptions, indicating loss of secondary structure. Curiously, when a CCL 95 °C heated-aliquot was cooled at 20 °C, and CD measurements were performed, it was observed a partial recuperation of secondary structure (Fig. 4B).

CD measurements in the far-UV revealed that sugars galactose, α-lactose, melibiose and lactulose caused distinct changes in the minimum and maximum absorption values (Fig. 4C).

3.7. Antibacterial activity and bacterial agglutination

CCL was able in agglutinate all strain cells tested, *S. aureus*, *S epidermidis* and *E. coli* (Fig. 5). However, CCL did not show inhibition of planktonic growth (data not shown).

3.8. Effect of CCL on biofilm formation

The results of total activity of CCL on biofilm formation are shown in Fig. 6. For total biomass quantification, CCL caused significant reductions in the total biomass in the highest concentrations tested, compared to the negative control (Fig. 6A, C and E). Regarding number of biofilm-associated cells, after contact of the adhered cells with CCL for 24 h no reduction in the number of cells was observed (Fig. 6B, D and F).

3.9. Effect of lactose on CCL antibiofilm activity

In general, the reduction of biofilm biomass caused for CCL was abolished in presence of 100 mM α-lactose (Fig. 7). As expected CCL at 250 μg mL^{-1} caused significate reduction of biomass biofilm of *S. aureus* and *S. epidermidis*. However, in presence of 100 mM α-lactose the activity of CCL on *S. aureus* was completely abolished, CCL incubated with lactose did not show significant difference compared with control (biofilm formed without CCL) (Fig. 7A). Regarding *S. epidermidis* biofilm, the activity of CCL was partially inhibited (Fig. 7B). Interestingly, the biomass biofilm of both species was increased in presence of 100 mM α-lactose.

4. Discussion

A new lectin from marine sponge *C. caribensis* was successfully purified by affinity chromatography on HCl-activated Sepharose.

Besides hemagglutinating activity, *C. caribensis* crude extracts showed strong hemolytic effect. After heat treatment (boiling of the extract at 100 °C for five minutes), hemolytic effect was not observed, but hemagglutinating activity remains. Galactose-containing carbohydrates (data not shown) weakly inhibited the lectin present in the boiled material, therefore, affinity chromatography was chosen to performer purification.

HCl-activated Sepharose was chosen because it is an excellent media for isolation of galactose-binding lectins, as reported elsewhere. [5,9,20,21].

Marine sponge are excellent sources of galactophilic lectins and therefore, several lectins have been isolated from marine sponges throughout affinity chromatography using galactose and its derivatives [5,22,23,24], including a galactose-binding lectin isolated from *Chondrilla nucula* (CN lectin) [25].

CN lectin and CCL shared some properties: 1) both lectins are homotetrameric, formed by four identical subunits linked by weak interactions; 2) they are stable at variation of pH and temperature and 3) they are glycoproteins. These lectins seem to diverge in an important aspect: the sugar specificity.

CCL was only inhibited by galactose-containing disaccharides, such as lactose and lactulose. Moreover, some *O*-linked glycoproteins composed of galactose, GalNAc and fucose residues [26] were able to inhibit CCL, while CN lectin was inhibited by monosac-

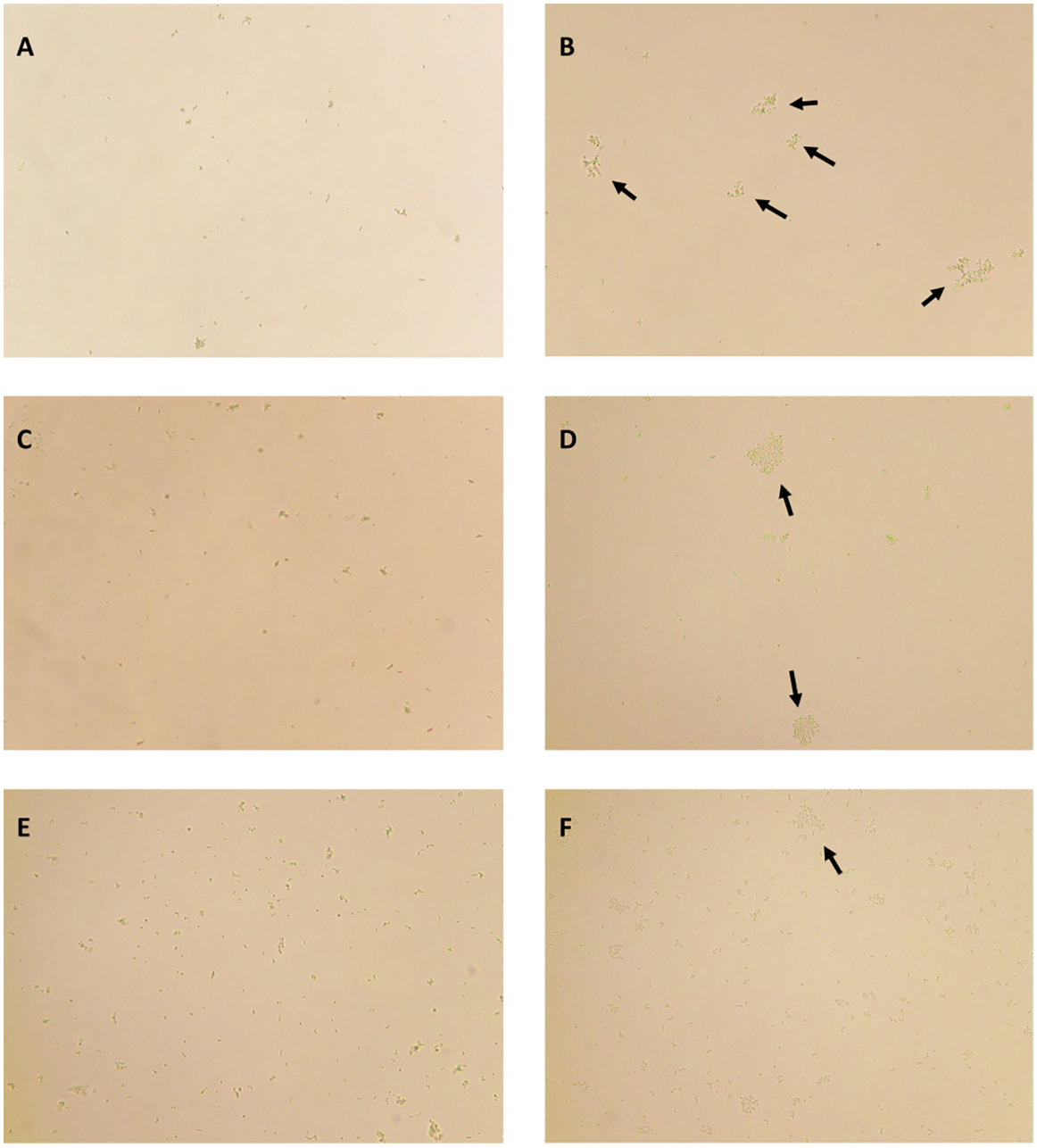

Fig. 5. Agglutination of Gram-positive and Gram-negative bacteria by CCL. S. aureus (A) S. epidermidis (C) and E. coli (E) cells incubated with TBS; S. aureus (B) S. epidermidis (D) and E. coli (F) cells incubated with CCL at (500 μg mL-1). Arrows indicate bacterial agglutination.

charaides, including galactose and GalNAc [25]. Recently, we have isolated a lectin from marine sponge *Aplysina lactuca* (ALL), which has similar inhibition profile that one showed by CCL [4].

Besides, resembling specificities ALL and CCL share similarity of amino acid sequence. Currently, ALL is an orphan lectin, but it showed slight identity and shares some common properties with lectin-I from marine sponge *Axinella polypoide* [4]. Possibly, ALL, lectin-I and CCL are members of a new lectin family, but to consolidate this family complete amino acid sequence of ALL and CCL is required.

The carbohydrates that inhibit CCL hemagglutinating activity showed high values of MIC, but, interestingly, some these sugars causes great changes in CCL tertiary structure, conform observed in CD measurements, suggesting that the interaction between CCL and carbohydrates may be deeper than the inhibition assay indicates. For instance, galactose showed no inhibitory effect in hemagglutinating inhibition assay, but its presence alters considerably CCL profile in CD measurement. On the other hand, lactulose caused inhibition of CCL, but showed slight effect on the CD assay.

Maximum values in far-CD were observed at wavelengths that aromatic amino acids usually absorb (*i.e.* 280 nm for Tryptophan, 260 nm for Tyrosine, and 240 nm for Phenylalanine). Modifications in the absorption of these wavelengths in the presence of sugars suggest that these amino acids are involved in carbohydrate recognition. In fact, several lectins have aromatic amino acids in their active sites; these residues directly interact with sugars by weak interactions [27,28].

Moreover, CD measurements helps us to understand the thermostability showed by CCL, which was just inactivated after boiling

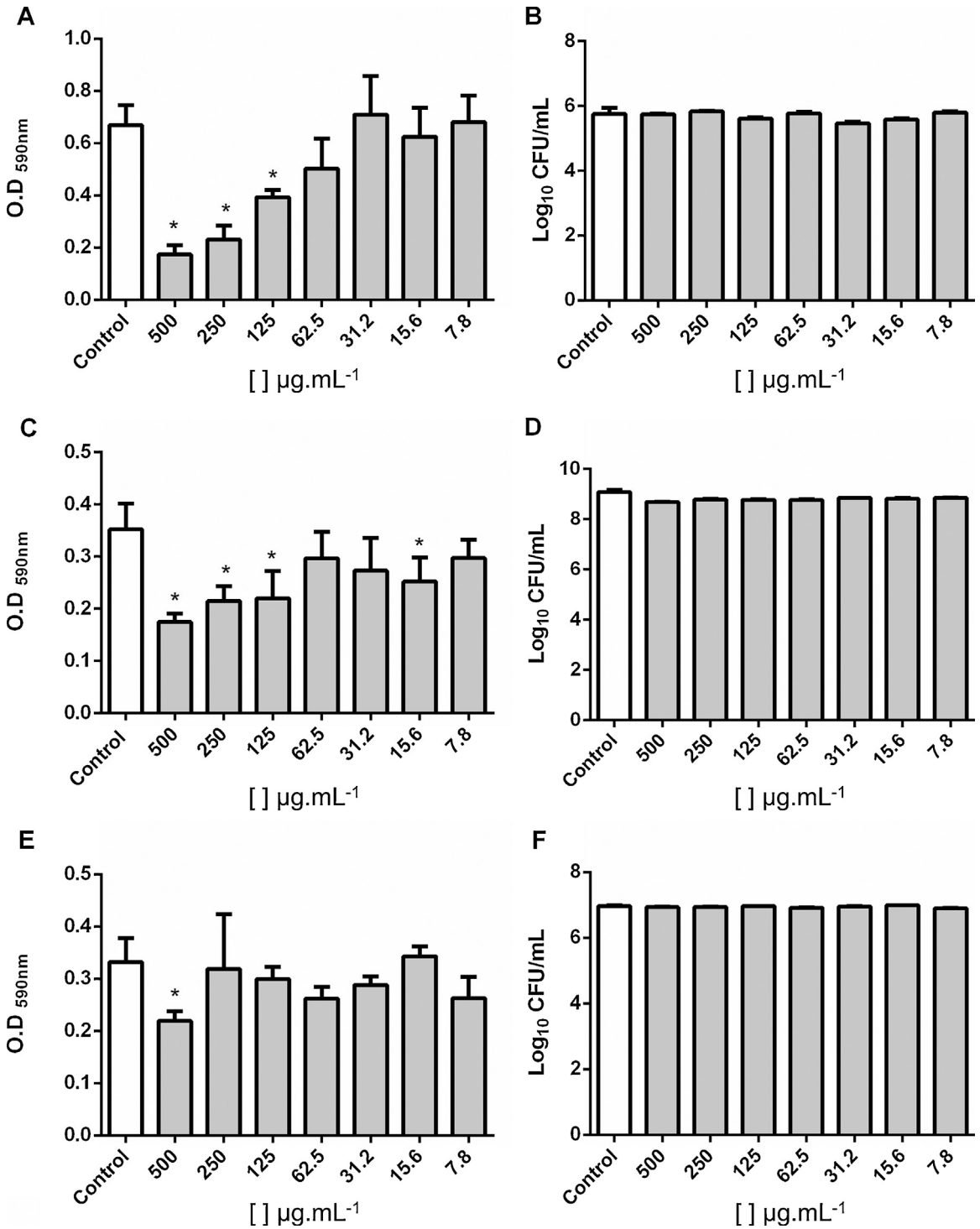

Fig. 6. Activity of CCL against biofilm formation (24 h) of Gram-positive and Gram-negative bacteria. Total biomass quantification measuring the intensity of crystal violet stain for *S. aureus* (A), *S. epidermidis* (C) and *E. coli* (E) biofilms; colony forming units per mL for *S. aureus* (B), *S. epidermidis* (D) and *E. coli* (F) biofilms. *Significantly different ($p < 0.05$) compared to the control group. Error bars display standard deviation (SDs) of the means.

for 1 h, whereas most lectins from marine source retain their activities in temperatures below 60 °C [14,29,30,31].

Commonly, protein stability is associated to some peculiar structural feature, such as disulfide bonds, but CCL possess no disulfide bond, second observed in ESI–MS. Then, it is possible that weak interactions compensate the absence of disulfide bonds through of the maintenance of a globular and compact structure even if the lectin was exposed to high temperatures. CCL is rich in β-conformations and few structures disordered were observed. Several lectins are rich in β-conformations, for instance the β-sandwich structure is probably the structure most commonly found in lectins. To our knowledge, few lectins showed β-conformations rates greater than CCL.

Sponge lectins have shown agglutination and potential antimicrobial activity against Gram-positive and Gram-negative bacteria [4,5,22,32]. In fact, some lectins may recognize and bind to glycans on cell surfaces, cell wall polysaccharides and interact with bacterial lipopolysaccharides (LPS) or with the extracellular matrix of

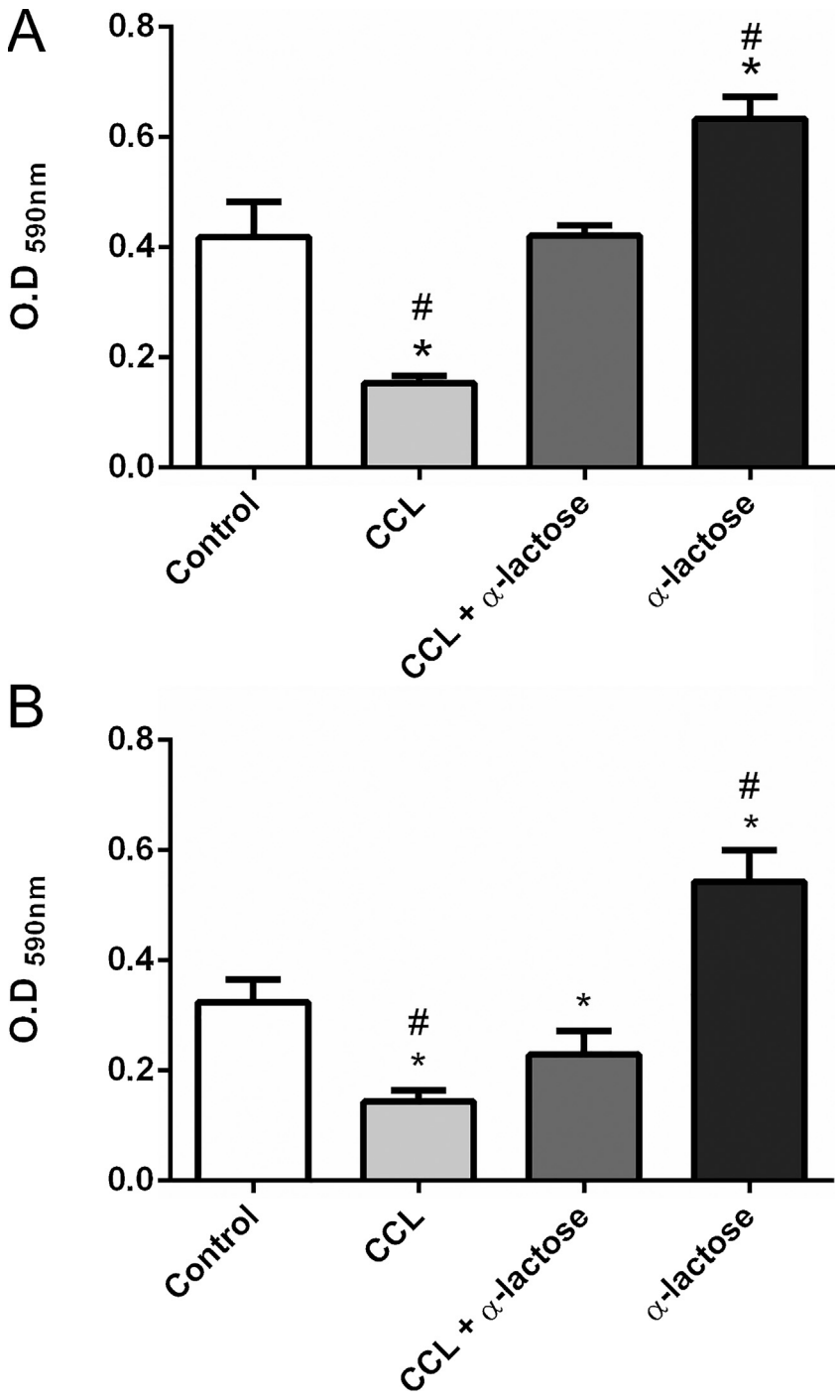

Fig. 7. Inhibitory effect of lactose on biomass biofilm reduction caused by CCL. Total biomass quantification measuring the intensity of crystal violet stain for *S. aureus* (A) and *S. epidermidis* (B) in presence of CCL (500 μg mL^{-1}); CCL (500 μg mL^{-1}) incubate with 100 mM α-lactose or 100 mM α-lactose alone. *Significantly different ($p < 0.05$) compared to the control group. # Significantly different ($p < 0.05$) compared to the CCL + α-lactose group. Error bars display standard deviation (SDs) of the means.

microorganisms [33–36]. Furthermore, according Gardères [1] the ability of sponge lectins to bind specific carbohydrates in bacterial cells could potentially be used to develop new antimicrobial agents.

In this study, CCL did not inhibited the planktonic growth, but caused agglutination of Gram-positive and Gram-negative bacteria. Others lectins have been similar activity [4,9]. ADEL caused agglutination in bacterial cells of *S. aureus*, however, did not inhibit the planktonic growth [9]. Interestingly, ALL agglutinated *S. aureus* and *E. coli* cells and was not able to inhibit the planktonic growth [4].

CCL showed a significant reduction of the total biomass on *S. aureus*, *S. epidermidis* and *E. coli* biofilms. However, corroborating with planktonic results, CCL did not reduced the viability of the biofilm-entrapped cells. Biofilms are described as microbial communities adhered to each other covered with a polymeric extracellular matrix produced by microorganisms themselves [37]. The biofilm resistance is reportedly up to 10 ± 1000 fold greater when compared with planktonic cells, protection conferred by biofilm matrix polymers [38]. The biomass of the biofilm consists of both bacterial cells and the biofilm matrix and in most biofilms

whereas the matrix can account for over 90% of the total biomass [39].

CCL activity was inhibited by α-lactose, suggesting that the CRD is involved in the antibiofilm activity. According Paiva et al. [40] the antibacterial activity of lectins occur due the specific recognition of components of the bacterial surface. Some studies reported that lectins could inhibit biofilm formation by interaction with bacterial cells constituents of the biofilm and alter the expression of genes associated with virulence and biofilm formation [16,41,42].

Thus, according to the results obtained in this study, reduction of the biofilm biomass caused by CCL can be considered a promising activity, considering that its effect could prevent the emergence of drug resistant strains, by the fact it does not eliminate pathogens directly.

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