Differential activity of a lectin from *Solieria filiformis* against human pathogenic bacteria

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Abstract

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A lectin isolated from the red alga *Solieria filiformis* was evaluated for its effect on the growth of 8 gram-negative and 3 gram-positive bacteria cultivated in liquid medium (three independent experiments/bacterium). The lectin (500 µg/mL) stimulated the growth of the gram-positive species *Bacillus cereus* and inhibited the growth of the gram-negative species *Serratia marcescens*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus* sp, and *Pseudomonas aeruginosa* at 1000 µg/mL but the lectin (10-1000 µg/mL) had no effect on the growth of the gram-positive bacteria *Staphylococcus aureus* and *B. subtilis*, or on the gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium.* The purified lectin significantly reduced the cell density of gram-negative bacteria, although no changes in growth phases (log, exponential and of decline) were observed. It is possible that the interaction of *S. filiformis* lectin with the cell surface receptors of gram-negative bacteria promotes alterations in the flow of nutrients, which would explain the bacteriostatic effect. Growth stimulation of the gram-positive bacterium *B. cereus* was more marked in the presence of the lectin at a concentration of 1000 µg/mL. The stimulation of the growth of *B. cereus* was not observed when the lectin was previously incubated with mannan (125 µg/mL), its hapten. Thus, we suggest the involvement of the binding site of the lectin in this effect. The present study reports the first data on the inhibition and stimulation of pathogenic bacterial cells by marine alga lectins.

Lectins are present in almost all living organisms. The main characteristic of this class of proteins is the ability to interact specifically and reversibly with carbohydrates and thus to combine with glyco-components on the cell surface (1). A number of possible physiological roles have been proposed for lectins, most of which are based on specific carbohydrate binding and recognition of the molecules and biological structures that contain these sugars (2). Lectins from terrestrial plants and animals have been isolated, characterized and studied extensively in many aspects of biochemistry and

biomedicine. In contrast, only a limited amount of information about lectins from seaweed is available, mainly due to difficulties in isolating and obtaining sufficient material for study (3). In general, algal lectins have a lower molecular mass than most higher plant lectins and have no affinity for simple sugars, being more specific for complex oligosaccharides, often glycoproteins (3). Most of the algae from which lectin has been obtained and purified belong to the Rhodophyta (4,5). Benevides et al. (4) purified and characterized the lectin from the red alga *Solieria filiformis* as having a low molecular mass of 29 kDa and agglutinating activity inhibited by the glycoproteins mannan, avidin, ovalbumin, and egg white.

Few reports have been published on the use of lectin or lectin-like substances from plants as inhibitors of fungal and bacterial growth (6-11). Yet, the concentration of these proteins offers great potential for differential cell recognition (12). Therefore, in this paper we report the action of the lectin from *S. filiformis* seaweed on the growth of 11 pathogenic species of bacteria.

The marine red alga *S. filiformis* (Kützing Gabrielson) was collected from Pacheco beach (Fortaleza, CE, Brazil). After collection, the material was separated from epiphytes, washed with distilled water and stored at -20ºC until use. Purification is described below.

Gram-positive bacterial strains of *Bacillus cereus*, *B. subtilis* and S*taphylococcus aureus*, and gram-negative bacterial strains of *Escherichia coli*, *Serratia marcescens*, *Salmonella typhi*, *S. typhimurium*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus* spp, and *Pseudomonas aeruginosa* were studied. Culture concentrations were adjusted by measuring turbidity at 630 nm of 10^3-10^4 colony-forming units (CFU)/mL.

The lectin from the marine red alga *S. filiformis* was purified following the protocol described by Benevides et al. (4). Algal material was ground to a fine powder in a mortar and pestle in the presence of liquid nitrogen. The powder was then stirred (1:3, w/v) with 20 mM Tris-HCl buffer (TB), pH 7.5, for 4 h at room temperature. Insoluble algal material was removed by filtration using a nylon membrane followed by centrifugation at 6000 *g* for 30 min at 4ºC. The crude, clear extract was submitted to treatment with solid ammonium sulfate (80% saturation) and the resulting precipitate (F 0/80) was recovered in TB and centrifuged at 16,000 *g* for 20 min at 4ºC. The clear supernatant was applied to an affinity chromatography on a Mannan-Sepharose 4B column (8 x 0.8 cm). The column was equilibrated with TB and unbound proteins were removed by elution with the equilibrium buffer. The retained lectin was then desorbed from the matrix by the addition of a 4.0-M urea solution to the gel. Fractions showing hemagglutinating activity were pooled, dialyzed against distilled water, freeze-dried, and assessed by discontinuous electrophoresis according to the method of Laemmli (13). A 12.5% polyacrylamide slab in 25 mM Tris-HCl, 0.2 M glycine, pH 8.9, with 0.1% sodium dodecyl sulfate was used. Sample and standards were prepared in 25 mM Tris-HCl buffer, pH 6.8, containing 1.0% SDS and 1.0% ß-mercaptoethanol. The picrate-Coomassie blue method, as described by Stephano et al. (14), was used to detect proteins. Hemagglutination assays were carried out using 2% trypsin-treated fresh rabbit erythrocytes (5). Proteins were estimated by the Bradford method (15) using bovine serum albumin as the standard.

Inhibition of bacterial growth was evaluated by determining the concentration of lectin that reduced culture growth compared to control according to the procedures described by Broekaert et al. (16). Ten microliters of the cultures was added to each well of 96-well microtiter plates (NUNC, Corning, NY, USA) containing nutritive broth (Merck, Darmstadt, Germany) with two-fold serial dilutions of the lectin (10-1000 µg/

mL). The plates were incubated at room temperature for a period of 48 h and turbidity was measured (20, 24 and 48 h) at 630 nm using a UV-visible spectrophotometer (Elx 800, Bio Tek® Inst., Inc., Winooski, VT, USA). The same assay was used to evaluate the action of the lectin on bacterial growth in the presence of its hapten. For this assay, the lectin was previously incubated with mannan at a concentration of 125 μg/mL at 37ºC for 1 h. The number of bacterial cells was obtained by counting viable colonies on the plates by the method of Collins et al. (17). All experiments were run in triplicate, and the averages of the readings, the standard errors and coefficients of variation were calculated.

Data were analyzed statistically by one-way analysis of variance (ANOVA) (General Linear Models in the GLM procedure) using MSTATC software, with the level of significance set at $P < 0.05$.

Purified lectin from the *S. filiformis* alga was prepared by extraction of fresh algal tissue in 20 mM Tris-HCl buffer, pH 7.5. The proteins were precipitated with 80% saturated ammonium sulfate (0-80% protein fraction), followed by affinity chromatography on a Mannan-Sepharose 4B column. The lectin preparation exhibited a single protein band with a molecular mass of 29 kDa on sodium dodecyl sulfate polyacrylamide gel and showed hemagglutinating activity against trypsin-treated rabbit erythrocytes (Figure 1).

The *S. filiformis* lectin at a concentration of 1000 μg/mL had an inhibitory effect on the growth curve of the gram-negative bacteria *S. marcescens* (growth curve not shown), *S. typhi*, *K. pneumoniae*, *E. aerogenes*, *Proteus* spp, and *P. aeruginosa* compared to control. This inhibitory effect significantly reduced ($P < 0.05$) the cell density of bacterial solutions at 24 h (21.6, 26.8, 27.4, 20.7, 18.9, and 24.5%, respectively) without any alterations in the profile of the growth curve (Figure 2A-E). A decrease in

the number of bacterial cells was detected by counting viable colonies on the plates when the bacterial suspensions of *E. aerogenes* and *P. aeruginosa* were incubated with the *S. filiformis* lectin (0.3×10^9 and 0.5×10^9 CFU/mL, respectively) and compared to the control (3.2×10^9 and 4.9×10^9 CFU/mL).

The inhibitory effect of the lectin on the growth of these bacterial species could be due to its interaction with the glycoconjugate mannan, its hapten, present on the surface of the cell walls of gram-negative bacteria. Strathmann et al. (18) observed that the lectin from *Canavalia ensiformis* seeds labeled with fluorescein isothiocyanate was capable of interacting with carbohydrates (hyaluronic acid, glucose, galactose, mannose, ribose, rhamnose, glucosamine, and galactosamine) of extracellular polymeric substances produced by the gram-negative bacteria *P. aeruginosa*. In contrast, the *S. filiformis* lectin had no effect on the growth
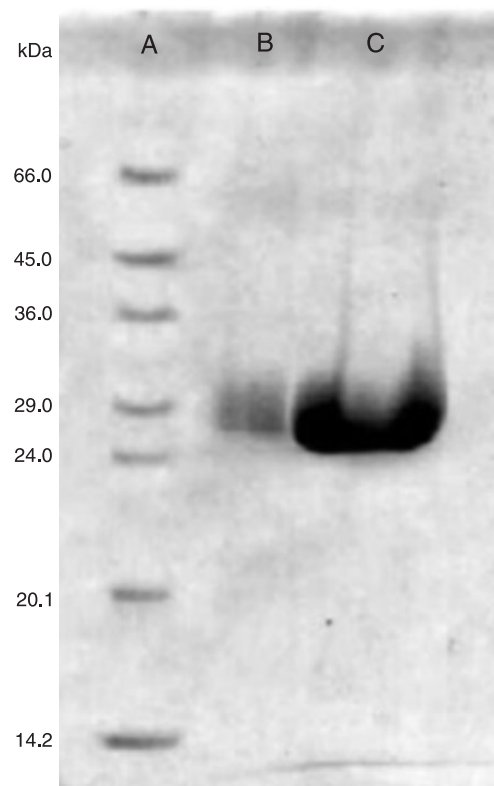

Figure 1. SDS-PAGE of a lectin purified from *Solieria filiformis*. A, Protein mass standards: 14.2-kDa α-lactoalbumin, 20.1-kDa trypsin inhibitor, 24.0-kDa trypsinogen, 29.0-kDa carbonic anhydrase, 36.0-kDa glyceraldehyde-3-phosphate dehydrogenase, 45.0-kDa ovalbumin, and 66.0-kDa bovine serum albumin. B and C, Purified lectin (5.0 and 20.0 μg, respectively).

of the gram-negative bacteria *E. coli* and *S. typhimurium* or on the gram-positive bacteria *B. subtilis* and *S. aureus* at any of the concentrations tested (data not shown). This might be due to the absence of specific carbohydrates on the surface of the cell walls of these bacteria or to inaccessibility of these carbohydrates to *S. filiformis* lectin. Indeed, red blood cells become more sensitive to agglutination by seaweed lectins when they

are treated with bromelain and trypsin, respectively (5,19), removing and/or exposing some of the membrane glycoproteins to facilitate bridge formation between cells without necessarily affecting the total number of lectin binding sites (20).

The lectin of *S. filiformis* at a concentration of 500 μg/mL had a statistically significant stimulatory effect ($P < 0.05$) only on the cell density of solutions of the gram-positive bacteria *B. cereus* at 24 h (78%) compared to control. This stimulatory effect was highest at a concentration of 1000 μg/mL (126%), demonstrating a dose-effect relationship (Figure 2F).

The inhibitory and stimulatory effects on bacterial growth were eliminated when the lectin was previously incubated with the hapten mannan, confirming the interaction of lectin with glycoconjugates present on the surface of the bacterial cell wall.

It is possible that the interaction of *S. filiformis* lectin with receptors on the surface of gram-negative bacteria promotes alterations in the flow of nutrients, thereby explaining the bacteriostatic effect.

An increase in the number of *B. cereus* cells was observed when the number of viable colonies on the plates containing the bacterial suspension incubated with the *S. filiformis* lectin (2.0 x 10^8 CFU/mL) was counted and compared to control (2.0 x 10^5 CFU/mL).

The fact that *S. filiformis* lectin had both stimulatory and inhibitory effects on the growth of pathogenic bacteria is intriguing and further studies are needed to evaluate the significance of the action of this protein on bacterial growth for clinical microbiology and possible therapeutic applications.
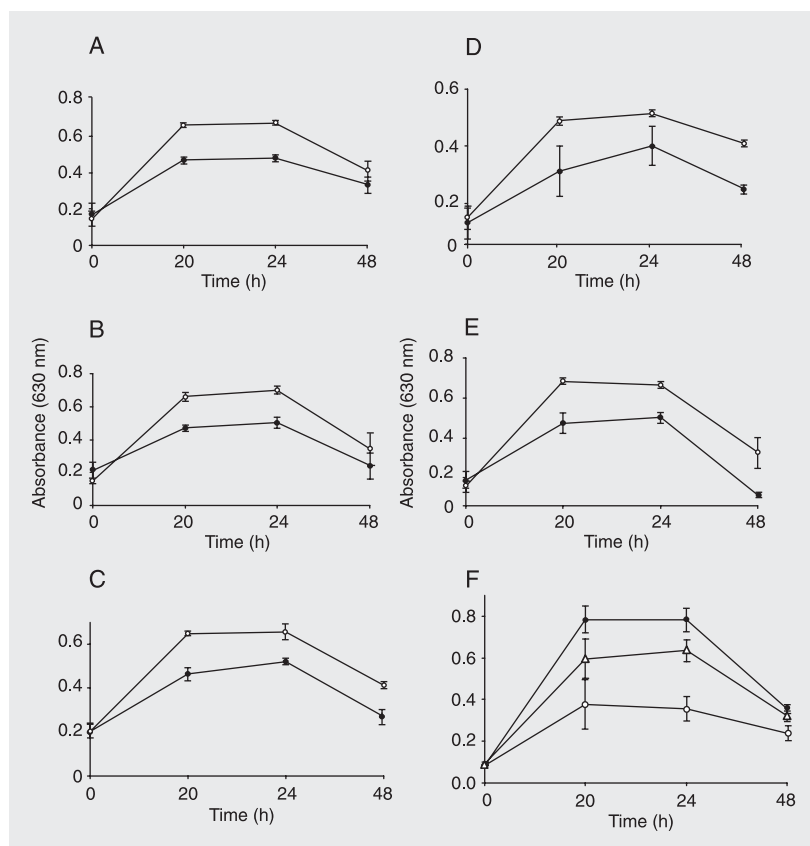

Figure 2. Effect of the *Solieria filiformis* lectin on the growth of gram-negative and gram-positive bacteria *Salmonella typhi* (A), *Krebsiella pneumoniae* (B), *Enterobacter aerogenes* (C), *Proteus* spp (D), *Pseudomonas aeruginosa* (E) and *Bacillus cereus* (F). Absorbance at 630 nm was used as a measurement of bacterial growth. Control (open circles), 500 μg/mL lectin (triangles), and 1000 μg/mL lectin (filled circles). Data are reported as the mean ± SD for three experiments.

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