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Biocontrol of *Fusarium* species by a novel lectin with low ecotoxicity isolated from *Sebastiania jacobinensis*

Antônio F.M. Vaz [a,*], Romero M.P.B. Costa [a], Ana M.M.A. Melo [b], Maria L.V. Oliva [c], Lucimeire A. Santana [c], Rosemeire A. Silva-Lucca [c,d], Luana C.B.B. Coelho [a], Maria T.S. Correia [a,1]

[a] Department of Biochemistry, Federal University of Pernambuco, Recife, Brazil
[b] Department of Biophysics and Radiobiology, Federal University of Pernambuco, Recife, Brazil
[c] Department of Biochemistry, Federal University of São Paulo, São Paulo, Brazil
[d] Center of Engineering and Exact Sciences, State University of Western Paraná, Paraná, Brazil

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ABSTRACT

A lectin from *Sebastiania jacobinensis* bark was isolated using a combination of acetone precipitation, ammonium sulphate fractionation, ion exchange and gel filtration chromatographies. The lectin purified, with a molecular mass of 52.0 kDa and composed of two subunits of 24 kDa, is a glycoprotein with a neutral carbohydrate content of 6.94%. The lectin shows maximum activity over the pH range 4.0–7.5 and heat stability up to 70 °C. Our results show that the lectin is an incompetitive inhibitor for trypsin, with a *Ki* of 0.39 ± 0.02 μM. Fluorescence spectroscopy indicated the existence of a hydrophobic surface. The percentages of secondary structure are 75% α-helix, 10% β-sheet, 5% β-turn and 10% unordered. Lectin inhibits the mycelial growth of *Fusarium moniliforme* and *Fusarium oxysporum* with an IC_{50} value of 123 ± 0.5 and 303 ± 0.9 μg, respectively. *Artemia salina* Leach and embryos of *Biomphalaria glabrata* are not affected by the lectin, indicating low environmental toxicity. Alternative viewpoints are presented that might hopefully help in future efforts to develop safer and more effective microbial control agents.

1. Introduction

Increasing awareness of the potential impacts of crop-protection agents or pesticide use has led to the development of research with natural products to ensure that risks to man and the environment are limited. Thus, the regulation of natural products as crop-protection agents should have to undergo the same procedure as for a conventional chemical product (Neale, 2000). Antimicrobial agents are provided for control of certain diseases of wheat and other cereals caused by *Fusarium* species, including *Fusarium* head blight of wheat and other cereals. These agents can also improve yield of wheat plants and cereals. Plant lectins are a heterogeneous group of proteins or glycoproteins that share the capacity to identify a specific carbohydrate. Their widespread distributions in the plant kingdom suggest a physiologically important function (Sharon, 2007). They have attracted great interest because of their various biological activities, such as antiproliferative, antitumour, antifungal and antiviral properties (Peumans & Van Damme, 1998). Seeds, especially of leguminous species, are common sources of lectins, but they are also present in latex and bark of different species (Branco et al., 2004; Wititsuwannakul, Rukseree, Kanokwiroon, & Wititsuwannakul, 2008). The proteins provide an opportunity for discovery and a starting point for optimising complex cellular processes and molecular mechanisms. Providing rigorous and comprehensive characterisations for these proteins is invaluable to researchers and frees them to confidently pursue creative experimentation. Circular dichroism (CD) spectroscopy can be a valuable method for determining the secondary structures of proteins (Johnson, 1999). Intrinsic fluorescence and 4.4′-Bis-1-anilinonaphthalene-8-sulphonate (Bis-ANS) have been used in folding, stability studies and as evidence of conformational change in proteins, by assessing hydrophobic regions (Hawe, Sutter, & Jiskoot, 2008).

The salt-water crustacean, *Artemia salina* Leach, is used as food for fish. *A. salina* is sensitive to the effect of active substances and its mortality is used to monitor toxicity because it is highly sensitive to many chemical substances (Almeida, Silva, & Echevarria, 2002). *Biomphalaria glabrata* – Say, 1818 – is a snail of the Planorbidae family with a wide distribution in Brazil. The ease of observing antibiotic effects of drugs on embryonic development of *B. glabrata* and mortality of *A. saline* has urged their use as a way to monitor the environmental impact and selectivity of microbiological control agents (Mclaughlin & Rogers, 1998; Münzinger,

* Corresponding author.
E-mail address: melo_vaz@ig.com.br (A.F.M. Vaz).
[1] Address: Av. Prof. Moraes Rego, 1235 – Cidade Universitária, Recife – PE – CEP: 50670-901, Recife, PE, Brazil. Tel.: +55 81 21268574; fax: +55 81 21268576.

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1987). Antifungal proteins have been isolated from a large number of plants and have been separated into many types comprising thaumatin-like proteins, chitinases, β-1.3-glucanases, thionins, plant defensins, ribosome-inactivating proteins, protease inhibitor-like proteins and lectins, which might hopefully help in future efforts to develop safer and valuable microbiological control agents for genetically modified plants.

Sebastiania jacobinensis Müll. Arg. (Euphorbiaceae family) is a common tree found in tropical regions of Brazil. The bark of this plant is popularly used against infections and hypersensitivity processes. In view of the benefits that microbiologically controlled agents with low environmental toxicity provide to vegetable biotechnology, we describe the isolation and partial characterisation of an antifungal lectin from the bark of *S. jacobinensis* and its ecotoxicological profile.

2. Materials and methods

2.1. Chemicals

Reference samples of 4.4′-Bis-1-anilinonaphthalene-8-sulphonate (Bis-ANS) were purchased from Molecular Probes Inc., USA. The broad-range standard marker proteins, sugar, glycoproteins, *N*-benzoyl-L-arginine-4-nitroanilide (L-BAPNA) and phenylmethyl sulfonyl fluoride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cercobin 700 WP (Thiophanate methyl) was purchased from Iharabras SA. (Sorocaba, SP, BR). All the solvents and other chemicals used were of analytical grade from Merck (Darmstadt, Germany). All solutions were made with water purified by the Milli-Q system.

2.2. Purification of *S. jacobinensis* bark lectin (SejaBL)

S. jacobinensis bark was collected from trees in the semi-arid region, state of Pernambuco, Brazil. *S. jacobinensis* bark powder was homogenised overnight at 4 °C in 10 mM Tris–HCl buffer (pH 8.5) with 0.2% (v/v) Triton X-100 detergent. The homogenate was centrifuged at 5000 g for 20 min (crude extract) followed by lyophilisation. A solution of acetone/water (4:1) was prepared and stored at −20 °C. In brief, two volumes of cold acetone were added to the crude extract (2:1), mixed well and kept on ice for 10 min. The solution was centrifuged at 10,000 g for 20 min at 4 °C. The supernatant was concentrated under vacuum until the total evaporation of acetone was achieved. The residual aqueous solution obtained was precipitated with $(NH_4)_2SO_4$ 60% of saturation. The precipitate was dialysed against the Tris–HCl buffer; this fraction was applied to a CM-Cellulose column (2 × 20 cm) previously equilibrated with the Tris–HCl buffer containing 150 mM NaCl. Following removal of unadsorbed proteins, the column was eluted with 1 M acetic acid. UV absorbance was used to monitor the elution and fractions (up to 0.100 abs) eluted with acetic acid were pooled, dialysed, concentrated by ultrafiltration (Amicon ultra-15, Mr 10,000 cut-off) and applied to a Sephadex G-100 column (2 × 70 cm) equilibrated with 150 mM NaCl. The column was eluted with the same solution at a flow rate of 0.5 ml min^{-1}. The first fraction eluted (SejaBL), after concentration by ultrafiltration in 10 mM phosphate buffer (pH 7.0), was applied to a Superdex 75 HR 10/30 column coupled to an ÄKTA purifier system (GE). The column was preequilibrated and eluted with 300 mM NaCl, at a flow rate of 0.5 ml min^{-1}, monitored by absorbance at 215 nm. The lectin was submitted to reverse-phase chromatography on a C-18 column performed on an HPLC system (Shimadzu) monitored at 215 nm, as described below. The total protein content of the crude extract and the purified lectin were determined by Lowry, Rosebrough, Farr, and Randall (1951), using the BSA standard curve, at a range of 0–500 μg ml^{-1}.

2.3. Hemagglutination activity and sugar specificity

Rabbit and human glutaraldehyde erythrocytes were obtained as described by Bing, Weyand, and Stavinsky (1967). Hemagglutinating activity (HA) defined as the lowest sample dilution showing hemagglutination was evaluated as described by Correia and Coelho (1995). Specific hemagglutinating activity (SHA) corresponded to the ratio between HA and protein concentration. The carbohydrate binding specificity of lectin was determined by HA inhibition using several sugars (D-glucose, *N*-acetyl-D-glucosamine, D-arabinose, D-mannose, L-fucose, L-raphinose, D-galactose, L-threalose, D-xylose, D-sucrose, L-rhamnose, L-celobiose, D-lactose, methyl-α-D-mannopyranoside and methyl-α-D-glucopyranoside) and glycoproteins (bovine serum albumin, casein, thyroglobulin, ovalbumin, fetuin and asialofetuin).

2.4. Effect of pH, temperature and metal ions

The effect of pH on HA was evaluated by incubating the lectin (0.5 mg ml^{-1}) at different pH values for 1 h at room temperature in selected buffers (10 mM citrate phosphate buffer, pH 4–7 and 10 mM tris–hydrochloric acid buffer, pH 8–11) and remaining SHA was determined in pH 7.0 at 25 °C. Heat stability was determined by incubation of lectin solution at different temperatures (30–90 °C for 30 min and 100 °C, 30–90 min) and remaining SHA was determined in pH 7.0 at 25 °C. The effects of Mg^{2+}, Zn^{2+} and Ca^{2+} were determined by incubation at the same volume of any metal ion (5, 10 and 20 mM) in 150 mM NaCl and lectin. An aliquot (50 μl) of the mixture was distributed in microtitre plate wells and the HA was analysed as described for the carbohydrate inhibition assay.

2.5. Molecular weight determination

Polypeptide chain analyses were performed after disulfid bridge reduction and alkylation. Previously lyophilised samples were reduced by Friedman reaction (Friedman, Krull, & Cavins, 1970) with some modifications as follows: the lectin (1.5 mg) was dissolved in 250 μl, 50 mM Tris–HCl, pH 8.6, 6 M urea, 10 mM EDTA, 179 mM DTT and incubated for 3 h at 37 °C, in the dark, before N_2 purging. The free sulphydryl groups were exposed to 100 μl iodoacetate and the reaction was continued for another 2 h under the same initial conditions. The iodoacetate derivative chains were desalted and separated on a C-18 column (Vydac-protein peptide ultrasphere) performed on a HPLC system (Shimadzu LC-10AD-kyto, Japan) and monitored at 215 nm. The column was equilibrated with solvent A (0.1% TFA in H_2O) and eluted using solvent B (90% acetonitrile: 10% H_2O: 0.1% TFA) in a non-linear gradient, where B = 5% at t = 5 min; B = 70% at t = 27 min; B = 80% at t = 60 min and B = 100% at t = 69 min. SDS–PAGE was carried out according to Laemmli (1970) and stained with a silver kit (Bio-Rad). Acid gel electrophoresis for native proteins was performed with Davis' system (1964). SDS–PAGE on reducing conditions was made after the Friedman reaction. Glycoproteins were detected by periodic acid-schiff (PAS) and estimation of carbohydrate content of the protein samples was done by the phenol sulphuric acid method, with a curve of D-mannose as a standard.

2.6. Fluorescence spectroscopy

Intrinsic fluorescence emission of the lectin solution (0.2 mg ml^{-1} in 10 mM phosphate buffer, pH 7.0) was measured at 25 °C using a spectrofluorimeter (JASCO FP-6300, Tokyo, Japan) in a cuvette (1-cm pathlength rectangular quartz). The excitation wavelength was 295 and 280 nm; emission spectra were recorded at a range of 305–450 nm and band passes were 5 nm. The

hydrophobic surface was determined using the same conditions by intrinsic fluorescence. The samples were transferred to the cuvette and then mixed with 5 μM Bis-ANS; fluorescence was measured in the JASCO spectrofluorimeter. The fluorescence emission obtained was of 400–600 nm with excitation at 360 nm.

2.7. Circular dichroism (CD) measurements

The CD spectrum was recorded over the range 195–250 nm, at 25 °C, on a Jasco J-810 spectropolarimeter using a cylindrical quartz cuvette of 1 mm pathlength with an average of 16 scans for protein solutions of approximately 0.02 mg ml^{-1} in PBS 10 mM, pH 7.0. The tertiary structure class analysis of lectin was performed by the Cluster program and their secondary structure analyses by the CDSSTR program.

2.8. Resistance proteases assay and trypsin inhibitory activity

Sensitivity of SejaBL to protease was developed according to Rios et al. (1996). 5.0 μg of α-chymotrypsin and trypsin in 0.1 M Tris–HCl buffer (pH 8.2) were preincubated for 3 h with 50 μl of SejaBL (0.5 mg ml^{-1}). Afterwards, the reaction was stopped with 5 μl of phenylmethyl sulfonyl fluoride (1.0 mg ml^{-1}). The sensitivity to protease was measured by lectin HA determination.

The determination of trypsin inhibitory activity was carried out according to the method of Lee and Lin (1995), by inhibition of hydrolysis of L-BAPNA in 0.1 M Tris–HCl buffer (pH 8.2) catalysed by trypsin. Four determinations were averaged for trypsin inhibitory activity and expressed as μg trypsin inhibited.

2.9. Antifungal activity

Assays of antifungal activity and quantitative assays to determine the inhibition concentration of 50% (IC_{50}) were performed as described by Wang and Ng (2007). The selected fungi, i.e., *Trichoderma viride* (URM-3344), *Fusarium oxysporum* (URM-2489), *Colletotrichum gloeosporioides* (URM-4911), *Fusarium moniliforme* (URM-3226), *Aspergillus niger* (URM-5238) and *Candida albicans* (URM-4388), stock obtained from Federal University of Pernambuco fungal cultures, were carried out in 60 mm petri plates containing 7 ml of potato dextrose agar (PDA). After the mycelial colony had developed, sterile blank paper disks (0.625 cm in diameter) were placed at a distance of 0.5 cm away from the border of the mycelial colony. A SejaBL aliquot (20 μl with 300 μg) was added to the disk. The positive control was Cercobin (2 μg) and negative control 150 mM NaCl. The plates were incubated at 23 °C for 72 h until mycelial growth had developed in the disks containing the negative control and had formed ring crescents of inhibition around disks containing samples with antifungal activity.

To determine the IC_{50} value, three concentrations of SejaBL (75, 150, and 300 μg) were added, separately, to three aliquots each containing PDA (4 ml) at 45 °C, mixed quickly and poured into three separate small petri dishes. After the agar had cooled down, a small amount of mycelia (same amount to each plate) was added. Buffer without SejaBL was used as control. After incubation at 23 °C for 72 h, the area of the mycelial colony was measured and the inhibition of fungal growth determined.

2.10. Environmental toxicity

A. salina (Brine Shrimp) encysted eggs (25 mg) were hatched in a beeker filled with seawater under artificial light at 30 °C, pH 8–9 in constant aeration. After 28 h the nauplii were collected with a pasteur pipette macroscopically and counted in the stem of a pipette against a lighted background. The nauplii were transferred to test tubes containing the samples. The lectin concentration ranged from 10 to 1000 μg ml^{-1} in vials containing 5 ml of seawater. Fifteen shrimp nauplii were added to each vial (45 shrimps per concentration). The plates were maintained under illumination. Survivors were counted after 24 h of incubation and the percentage of deaths at each dose and control was determined. The bioassay was performed as described previously by Meyer et al. (1982) and developed with minor modifications. *B. glabrata* embryos (0–15 h after spawning) were exposed for 48 h to SejaBL (10–200 μg ml^{-1}) and the embryo development was followed for an additional 10 days in the absence of further exposure. The assay was carried out according to Oliveira-Filho and Paumgartten (2000).

2.11. Statistical analysis

Data are represented as mean ± SEM. The antifungal activity IC_{50} values and their 95% confidence intervals (CI 95%) were obtained by non-linear regression using the GraphPrism® (GraphPad Software Inc., San Diego, CA, USA). The lethal concentration of 50% (LC_{50}) for brine shrimp and *B. glabrata* embryos was obtained from 24 and 48 h, respectively; the percentage of deaths were estimated using the Probit method, a parametric statistical procedure for estimating the LC_{50} and the associated 95% confidence interval, described by Finney (1971).

3. Results and discussion

The lectin was extracted successfully with 0.2% Triton X-100 present in the solution. Only one bioactivity peak was detected with acetic acid elution from a CM-Cellulose column (Fig. 1A) and a Sephadex G-100 column (Fig. 1B) with specific hemagglutinating activity (SHA) of 8192 (Table 1). Superdex 75 gel filtration (Fig. 1C) showed an apparent molecular weight of 50 kDa which was eluted on a C-18 column (Fig. 1D) with 70% of acetonitrile. Analysed by SDS–PAGE, SejaBL showed a single band of 52 kDa (Fig. 2A) and, after the Friedman reaction, showed a dimeric protein composed of two subunits with the same molecular mass of 24 kDa and another fragment of less than 6 kDa (Fig. 2B). After desalting on a C-18 column, the two chains showed different mobility (Fig. 2C), suggesting that the chains are covalently connected by disulfide bonds. In general, the molecular mass of subunits back lectin is around 30 kDa, with the fully active lectin forming either dimers or tetramers. Molecular masses ranging from several kilodaltons to about 67 kDa have been reported for antifungal proteins (Ng, 2004).

The lectin is an acidic protein (Fig. 2D) with pH stability ranges of 4.0–7.5 (Fig. 3A). The lectin band was weakly stained by PAS, and a positive phenol–sulphuric acid assay revealed a glycoprotein with 6.94% covalently linked carbohydrates. The native active form was heat-stable up to 70 °C. A small decrease in activity was observed when the temperature was raised from 70 to 100 °C (Fig. 3B). SejaBL agglutinated erythrocytes from rabbits and humans of all blood groups except B. Fetuin, asialofetuin, bovine serum albumin and casein inhibited SejaBL induced rabbit erythrocyte agglutination, whereas monosaccharides did not. Other lectins described have also shown wide specificity to glycoproteins and revealed plasticity in the carbohydrate-binding region (Rego et al., 2002; Sultan, Kenoth, & Swamy, 2004). Ions did not affect HA and the lectin did not suffer apparent hydrolysis by proteases keeping HA. However, our results show that SejaBL is an incompetitive inhibitor for the trypsin, with a *Ki* of 0.39 ± 0.02 μM (Fig. 4). Trypsin-inhibitor proteins are generally found in vegetative tissues and are believed to play a role in defence against predators (Chen, Brown, Russin, Lax, & Cleveland, 1999).

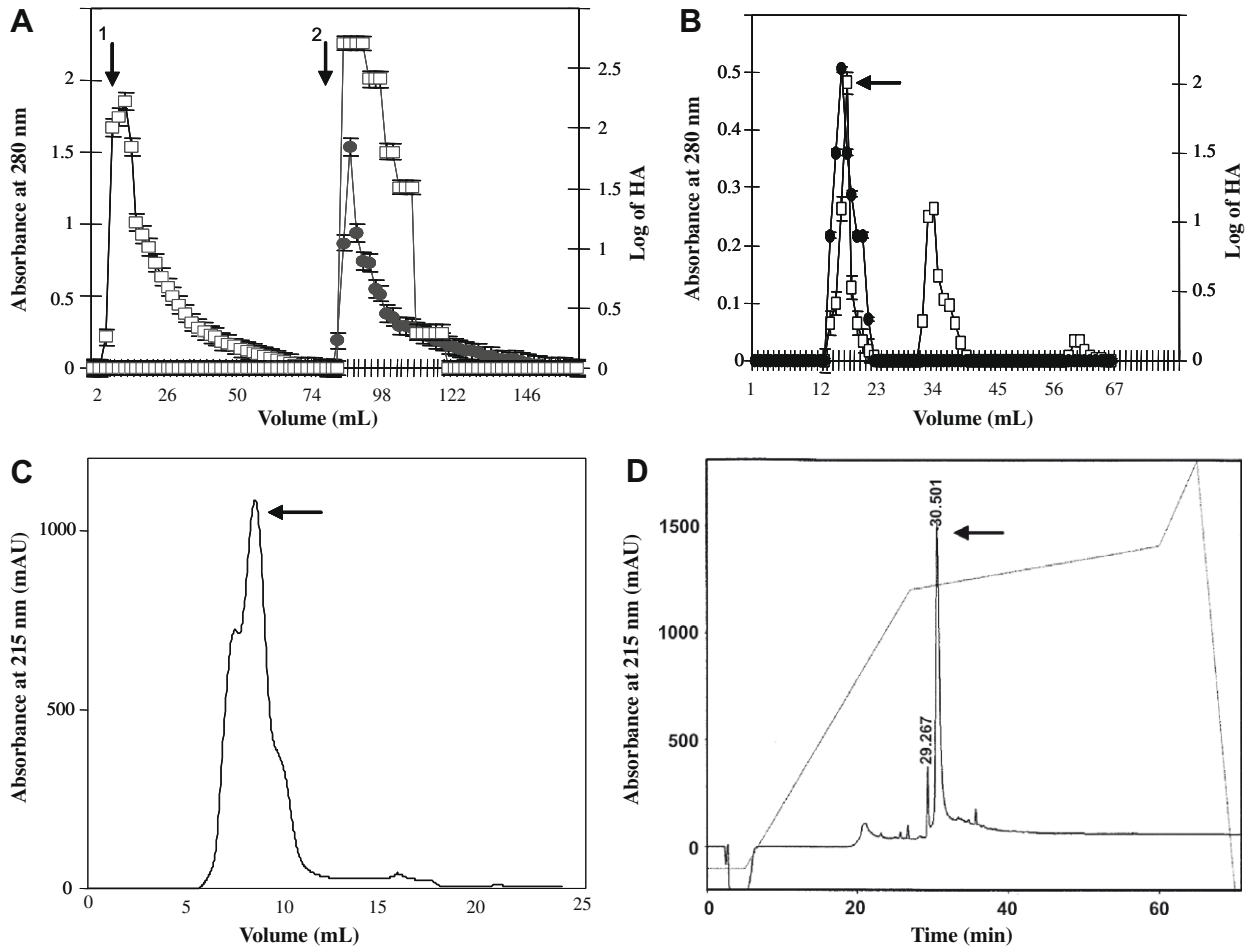

Fig. 1. (A) Purification by ion exchange (40 mg of protein). Arrows indicate elution with Tris–HCl buffer (1), followed by 1 M of acetic acid (2). Absorbance at 280 nm (-□-); log of HA (-●-). (B) Gel filtration in Sephadex G-100. Absorbance at 280 nm (-□-); log of HA (-●-). (C) Superdex 75 column coupled to an ÄKTA purifier system. (D) Reverse-phase chromatography in a C-18 column of a HPLC system.

Table 1
Yields and hemagglutinating activities of various fractions (from 100 g powdered *S. jacobinensis* bark).

Sample	Yield (mg)	Specific hemagglutinating activity (units/mg)	Total hemagglutinating activity (units)	Recovery of hemagglutinating activity (%)	Folds of purification
Crude extract	14,400	284.4	4.09×10^6	100	1
$(NH_4)_2SO_4$ fraction	1323.2	3951	5.22×10^6	127.67	13.9
CM-Cellulose	96	6827	6.55×10^5	16.01	24
Sephadex G-100	20	8192	1.63×10^5	4	28.8

The intrinsic protein fluorescence spectra of SejaBL (Fig. 5A) revealed one major peak at 330 nm, indicating the presence of tryptophan in a highly hydrophobic region. Interestingly, Bis-ANS fluorescence observed and compared with buffer indicates a hydrophobic surface turned to the solvent (Fig. 5A). Prasad, Ludueña, and Horowitz (1986) suggested that the immediate increase in Bis-ANS fluorescence intensity observed in proteins is due to the binding at primary hydrophobic site(s). Steadman et al. (2004) noted that a hydrophobic surface produced decreasing stability and increasing structural alteration in the protein after chemical or physical stress. The lower agglutination activity, at the basic pH values and high temperature might be due to the hydrophobic character of SejaBL.

The tertiary structure class of the protein can be related to the CD spectrum. In principle the CD spectra can distinguish four classes: all-α, all-β, α+β, α/β. The CD spectrum of SejaBL (Fig. 5B) showed two negative CD bands, one at 222 nm and one at 208–210 nm, and one positive band near 195 nm. The Cluster analysis showed that SejaBL belongs to the α+β class. Usually, for α+β proteins the 210 nm band has a larger intensity than the 222 nm band, this characteristic can be observed in the CD spectrum of SejaBL (Venyaminov & Yang, 1996). The percentages of secondary structure for SejaBL were 75% α-helix, 10% β-sheet, 5% β-turn and 10% unordered with a root mean square lower than 1%.

Among the fungi tested, SejaBL exerted antifungal action against *F. moniliforme* and *F. oxysporum* but not against *T. viride*, *C. gloeosporioides*, *A. niger* and *C. albicans*. The IC_{50} values of its antifungal activity towards *F. moniliforme* and *F. oxysporum* were, respectively, 123 ± 0.5 and 303 ± 0.9 μg (Table 2A), similar to those previously reported for antifungal lectins (Sitohy, Doheim, & Badr, 2007), and some are linked to protease inhibitory activities (Kim et al., 1999). The different sugar-binding specificities of lectins to phytopathogenic fungi were revealed recently by fluorometric study (Khan, Ahmad, & Khan, 2007) showing that specificity is a

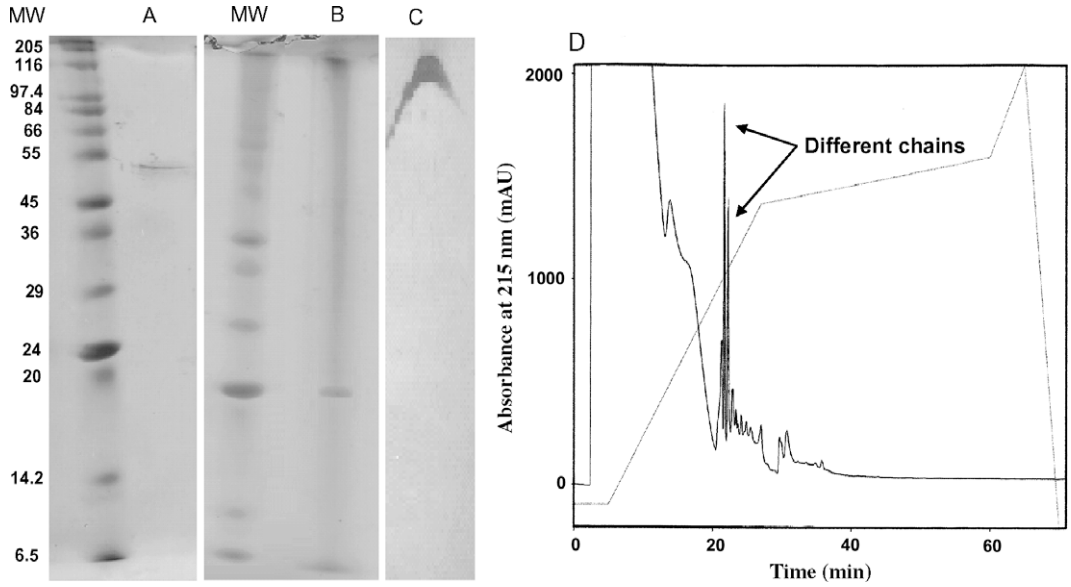

Fig. 2. SDS–PAGE was performed in a discontinuous system with 10% separating and 5% stacking gels (A) Molecular weight determination of SejaBL. (B) After Friedman reaction. (C) Electrophoresis under native conditions for acid protein. (D) Chains separations. The following standard marker proteins were used. Rabbit muscle myosin (205 kDa), *E. coli* β-galactosidase (116 kDa), rabbit muscle phosphorylase b (97.4 kDa), rabbit muscle fructose-6-phosphate kinase (84 kDa), bovine serum albumin (66 kDa), bovine liver glutamic dehydrogenase (55 kDa), egg albumin (45 kDa), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 kDa), bovine erythrocyte carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), bovine milk α-lactalbumin (14.2 kDa) and bovine lung aprotinin (6.5 kDa).

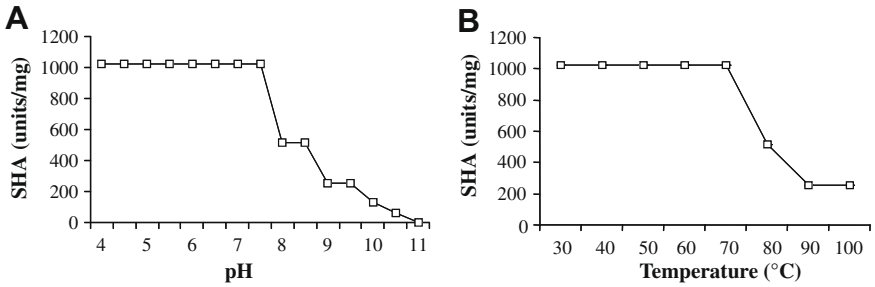

Fig. 3. Effects of temperature and pH on SHA. SHA in NaCl 150 mM with rabbit erythrocytes was 3.01 in pH 7.0 at 25 °C.

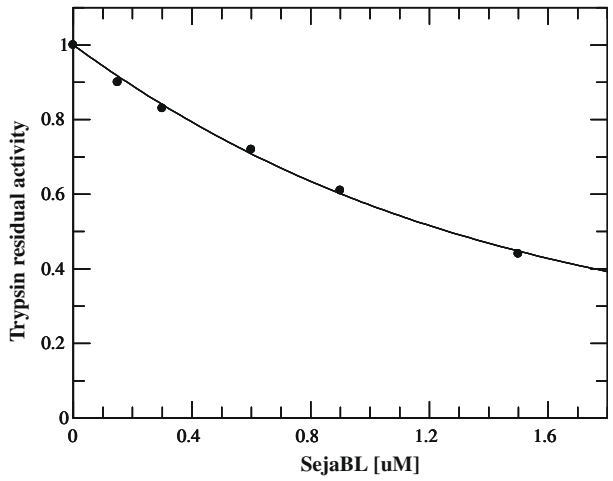

Fig. 4. Inhibition of purified SejaBL against trypsin. The assay for the inhibition of trypsin was carried out using 4.2 μM porcine pancreas trypsin.

criterion of selective action. In an extensive study, 11 purified lectins representing the major carbohydrate specificity groups were all found to cause growth disruption during germination of spores in fungal phytopathogens (Brambl & Gade, 1985). *Fusarium* species are pathogens of bananas, corn and rice. Damage produced by these fungi cause loss of cereals with harmful effects on human health and agriculture. The use of synthetic antifungal agents has caused problems of toxicity and aquatic environmental contamination (Canton, 1976).

The lectin showed percentage deaths of *A. salina* with LC_{50} of 715.89 ± 1 μg ml^{-1} (Table 2B). Lectin did not present toxicity in blastulae and embryo development of *B. glabrata*. Lethality was not observed until 200 μg ml^{-1} (Table 2B). This data suggests that antifungal activity is unrelated to lytic action or membrane instability. The inhibitory effect on embryo development was not observed after the first cleavage, and the cells presented nuclear organisation and homogeneous cytoplasm, with no impairment of DNA or protein synthesis (Fusetani, 1987). In order to establish the toxicity of new natural products, some tests have been used as an assay of lethality. *A. salina* and embryo development of *B. glabrata* was developed to detect bioactive compounds in plant extracts (Nick, Rali, & Sticher, 1995) but may also be used to express the toxicity of a compound active against non-target organisms, expanding the sensitivity to other organisms in the food chain such as small fish and amphibians (Lima et al., 2002; Oliveira-Filho & Paumgartten, 2000).

Some *Fusarium* species produce mycotoxins in cereal crops that can affect human and animal health (Hesseltine, 1974). SejaBL,

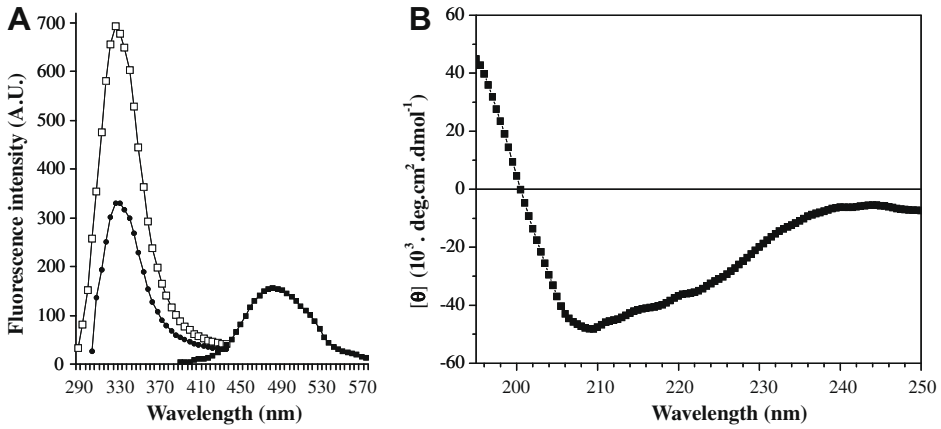

Fig. 5. (A) Intrinsic fluorescence emission of SejaBL, excitation at 280 nm (□) and 295 nm (●). Bis-ANS fluorescence, excitation at 360 nm (■). (B) CD spectrum of SejaBL. The spectrum was recorded over the range 195–250 nm, at 25°, in 1 mm cell pathlength. The CDSSTR program was used for estimation of secondary structure of SejaBL. The calculated fractions were 75% α-helix, 10% β-sheet, 5% β-turn and 5% unordered.

Table 2
Linearity value for the antifungal activity (A) and environmental toxicity (B).

	Antifungal activity	IC_{50} ± SD	Regression equation	Coefficient of determination $(r^2)^2$
A	*F. moniliforme*	123 ± 0.5 μg	$y = 0.1955x + 4.386$	0.992
	F. oxysporum	303 ± 0.9 μg	$y = 0.0741x$	0.99
	Toxicity environmental	LC_{50} ± SD	Regression equation	Coefficient of determination $(r^2)^2$
B	Embryo of *B. glabrata*	Nontoxic	–	–
	A. salina Leach	715.89 ± 1 μg/mL	$y = -0.1799x + 178.79$	0.983

compared with the reference chemical fungicides (Bollen, 1972), presents a higher degree of selectivity, does not affect conservation of and access to biological diversity by low environmental contamination. Moreover, the use of intrinsic biological control agents in plants can be a cheap alternative and prevent pollution of the environment as they are biodegradable. Thus, the isolation of a plant lectin with inhibitory activity toward fungi and with low environmental toxicity has important applications in vegetable biotechnology as a promising biological control agent in genetically modified plants.

In conclusion, this study supports the optimistic prospects of a lectin containing low environmental toxicity and inhibition of *Fusarium* species when evaluated using an *in vitro* model. Therefore, these results suggest that food rich in this biological control agent produced by genetically modified plants may be as efficient as synthetic antifungal agents (e.g., Cercobim) in reducing the quantity of contaminated cereals with harmful effects on human health. In addition, application of this finding to the agropecuary industry might generate more profitable and productive harvests without damaging the environmental biodiversity.

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