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ANTIFUNGAL ACTIVITY OF LECTINS AGAINST YEAST OF VAGINAL SECRETION

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ABSTRACT

Lectins are carbohydrate-binding proteins of non-imune origin. This group of proteins is distributed widely in nature and they have been found in viruses, microorganisms, plants and animals. Lectins of plants have been isolated and characterized according to their chemical, physical-chemical, structural and biological properties. Among their biological activities, we can stress its fungicidal action. It has been previously described the effect of the lectins Dviol, DRL, ConBr and LSL obtained from the seeds of leguminous plants on the growth of yeasts isolated from vaginal secretions. In the present work the experiments were carried out in microtiter plates and the results interpreted by both methods: visual observations and a microplate reader at 530nm. The lectin concentrations varied from 0.5 to 256µg/mL, and the inoculum was established between 65-70% of trammitance. All yeast samples isolated from vaginal secretion were evaluated taxonomically, where were observed macroscopic and microscopic characteristics to each species. The LSL lectin did not demonstrate any antifungal activity to any isolate studied. The other lectins DRL, ConBr and DvioL, showed antifungal potential against yeast isolated from vaginal secretion. These findings offering offer a promising field of investigation to develop new therapeutic strategies against vaginal yeast infections, collaborating to improve women's health.

Key words: Yeast, sensitivity, lectins

INTRODUCTION

Lectins are proteins or glycoproteins belonging to the immune system that bind specifically and reversibly to mono- and oligosaccharides in the form of free or glycoconjugates (glycoproteins and glycolipids). Thus, combined with biological molecules and structures containing these sugars without altering the structure of the glycosidic bonds in

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covalent binding sites (11, 28, 37).

They are found widely in animals, plants, fungi, bacteria and viruses (11, 24, 29, 33) but these substances are present in greater quantities in grain legumes and grasses (1, 9, 28, 41). The seeds of legumes such as beans and peas are rich sources of lectins which have been extensively characterized in relation to chemical properties, physical chemistry, structural and biological (8, 27, 28, 31). The parts of plants more susceptible to attack by predators, such as seeds, contain lectins that are in highest concentration. With the exception of chitinases, glucanases and glycosidases, lectins are the only proteins that recognize and bind to glycoconjugates present on the surface of bacteria and fungi or exposed in the intestinal tract of insects and mammals (33, 41).

Dioclea violacea, *D. rostrata* and *Canavalia brasiliensis* are legumes which are extracted lectins Dviol, DRL, ConBr respectively, which have affinity for glucose and mannose (3, 8, 10). These lectins showed induction of histamine in mice (19), *in vivo* protective effect against *Leishmania amazonensis* infection in BALB/c (6), lymphocyte stimulation in humans (5), stimulating the production of macrophages and lymphocytes in C3H / Hej (34).

Lonchocarpus sericeus is a legume which is extracted with LSL lectin affinity for N-acetylglucosamine (31). As demonstrated antiinflammatory activity in cases of peritonitis in mice, marked reduction of bacteria that colonized this region (1) and inhibition of neutrophil migration in inflammatory processes (31).

Some studies have reported the antifungal activity of lectins from leguminous plants such as bean *Phaseolus vulgaris* (8); fruit as pitomba *Talisia esculenta* (18), jackfruit *Artocarpus integrifolia*, breadfruit *A. incisa* (35) and soursop *Annona muricata* (16), fungi such as *Kluyveromyces bulgaricus* (36), and *Schizophyllum commune* (13), nettle *Urtica dioica* (7), potato (20), wheat germ (14) and Chinese herb *Astragalus mongholicus* as (40). These lectins are called, respectively: lectins from *Phaseolus vulgaris*, TEL, "jackin", "frutackin, AML, Kb-CWL I, SCL, UDA, potato lectin, WGA, AMML.

The susceptibility to antifungal activity of lectins has been observed in yeast as *Sacchromyces cerevisiae* (18, 35, 36), *S. bayanus, S. uvarurn, Candida albicans, C. glabrata, Rhodotorula mucilaginosa, Pichia pastoris, Kluyveromyces bulgaricus, K. lactis, Schizosacchamyces bulgaricus, S. octosporus* (36), and filamentous fungi such as *Trichoderma viride, T. hamatum* (7), *Coprinus comatus, Rhizoctonia solani* (41), *Fusarium oxysporum* (14, 16, 40, 41), *Colletorichum lindemuthianum* (18) *F. moniliforme* (35) *C. musae, F. solani* (16), *Aspergillus niger* (13), *Phoma betae*, *Phycomyces blakesleeanus, Septoria nodorum, Phytophthora erythroseptica* (7) *F. graminearum* (14) *Colletorichum* sp. *Drechslera Turkish* origin (40), *Botrytis cincerea* (7, 40).

However, other lectins have no antifungal activity (30, 37, 38, 39, 42) and some showed activating effect on the kinetics of growth of the fungus (22, 32, 36).

This study aimed to determine the antifungal effect of lectins from leguminous seeds on yeasts isolated from vaginal secretions.

MATERIALS AND METHODS

Yeasts

The yeasts were isolated from vaginal secretions of pregnant and non-pregnant women with and without symptoms, for investigation of vulvovaginal yeast infections.

The patients were from the gynecology, pediatrics and medical clinic. The vaginal secretion samples were collected between the months of October 2003 and August 2004, the Center for Health Manuel de Araújo Caldas, located in the neighborhood of Arthur Lundgren I in the City of Paulista, Pernambuco. We used 30 samples belonging to the genera *Candida, Rhodotorula, Trichosporon* and *Kloeckera.* All yeasts are deposited in the fungal collection - URM, Department of Mycology, Federal University of Pernambuco and were

subjected to taxonomic confirmation according to the authors (4, 23, 25) which are based on morphological and physiological evidence. The project was approved by the Ethics in Research of the Science Center Health, Federal University of Pernambuco, 304/2003-CEP/CCS Protocol.

Sample Reactivation

The stocked samples preserved in mineral oil were reactivated by growth in glycoside broth and then transferred to slants containing Sabouraud agar plus yeast extract (SAB+YE) (12).

For confirmation of the identification: Bovine bile water medium, C and N basic medium and sugar fermentation medium. For species confirmation, the classical methods of taxonomy of yeasts were utilized (4, 23, 25).

Procedures for assessing the antifungal activity of lectins

Culture medium: The culture medium used for the procedure was RPMI 1640 (Sigma-Aldrich, USA) with L-glutamine and without sodium bicarbonate, buffered to pH 7.0 with morfolinepropanesulfônico acid (MOPS) to 0165M (Sigma-Aldrich, USA). The medium was sterilized by membrane filtration with porosity of 0.22 µm (Millipore).

Lectins: We used four lectins extracted from legumes, such as Dviol, DRL, ConBr and LSL, respectively extracted from *Dioclea violacea, D. rostrata, Canavalia brasiliensis* and *Lonchocarpus sericeus*. The first lectin was obtained from seeds of legumes from Rio Grande do Sul (Brazil) and the rest of the state of Ceará (Brazil). These lectins were purified (2, 8, 26, 28) and kindly provided by the Laboratory of biologically active molecules from the Federal University of Ceará. After solubilization in sterile distilled water were prepared ten concentrations between 0.5 and 256µg/mL, followed by serial dilutions to achieve the final concentration 10 times higher than the concentration used in the procedure. Later, they were diluted (1:50) in RPMI 1640 to obtain the concentration of two times higher than necessary.

Preparation of inoculum

The inoculum were standardized according to the procedure adopted by the CLSI (15), where there was a peal of the microorganisms in tubes containing Sabouraud dextrose agar at room temperature (RT =28° C ± 1°C).The inoculum was prepared by selecting five colonies with a diameter of about 1mm to 24 hours of culture. The colonies were suspended in 5ml of sterile distilled water, where the resulting suspension was placed in a vortex shaker for 15 seconds and the cell density was adjusted in a spectrophotometer by adding sterile distilled water sufficient to obtain the equivalent transmittance of a standard solution of McFarland 0.5, at a wavelength of 530nm. This procedure provides a standard yeast suspension containing 1 x 10^6 a 5 x 10^6 cells per mL. The suspension of work is produced by making a 1:100 dilution followed by a 1:20 dilution of the standard suspension with RPMI 1640 liquid medium, resulting in a concentration of 5,0 x 10^2 a 2,5 x 10^3 cells per mL^2.

Procedure for evaluation of antifungal activity

The microtiter method in liquid medium was performed in accordance with the document M27-A2 (15) in sterile microtiter plates with lids (TPP, Switzerland) containing 96 wells with U-shaped (flat bottom) were placed 100 mL of lectins in rows 1 to 10, with each concentration was placed in a row. In the wells of rows 11 and 12 were placed 100µL of RPMI 1640, which were the growth control and sterilization of the medium, respectively. These plates were stored at -20°C until use.

In due course, was deposited in each well 100µL of standardized inoculum, as previously mentioned, and the microtiter plates were incubated at room temperature (RT = 28°C ± 1°C) for seven days for the interpretation of results.

Data analysis

Every experiment was performed in duplicate. The concentrations inhibitory and fungicidal (MIC and MFC) of

each sample, geometric mean inhibitory concentrations and minimal fungicidal (GMMIC and GMMFC) determining the concentration able to inhibit 50% or 100% of samples (MIC_{50} and MIC_{100}, respectively).

Statistical analysis consisted of presentation of events in absolute numbers, percentages and averages, and used the Fisher exact test for the association of variables showing significance ($p < 0.05$).

Determination of minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

The determination of minimum inhibitory concentration of each sample was performed by visual observation of each well that showed a reduction of fungal growth and by reading the microtiter plates by the Microplate Manager program, model BenchMark Plus (Bio-Rad Laboratories Inc.) at 530nm. Taking into account the total growth (100%) in the control well, the percentage reduction in growth was attributed to the remaining wells. In microdilution plates after incubation (RT = 28°C ± 1°C), we observed the presence or absence of visible growth.

RESULTS

All yeast strains isolated from vaginal secretions were evaluated taxonomically, which were observed macroscopic and microscopic characteristics relevant to each species. The species of yeasts isolated from vaginal secretions tested in this study are shown in (Table 1).

Table 1. Yeast strains isolated from vaginal secretion from October 2003 to August 2004.

Registration URM Culture Collection	Species
4987	*Candida albicans*
4990	*C. albicans*
4986	*C. albicans*
4979	*C. azyma*
4985	*C. geochares*
4975	*C. guilliermondii*
4976	*C. marítima*
4983	*C. membranaefaciens*
4982	*C. obtusa*
4984	*C. parapsilosis*
4970	*C. parapsilosis*
4972	*C. robusta*
4974	*C. sake*
6088	*C. salmanticensis*
4978	*C. shehatae*
6084	*C. tropicalis*
6090	*C. tropicalis*
6089	*C. tropicalis*
4989	*C. tropicalis*
4981	*C. tsuchiyae*
4980	*C.versatilis*
5002	*Kloeckera apiculata*
5092	*Rhodotorula glutinis*
6085	*R. minuta*
6086	*R. minuta*
6087	*R. minuta*
4971	*R. graminis*
4988	*R. pallida*
6083	*R. rubra*
4973	*Trichosporon cutaneum*

The DRL lectin showed antifungal activity in four isolates, with geometric mean concentrations of 128µg/mL in *C. guilliermondii* (URM4975) and 4µg/mL in *C. shehatae* (URM4978), 64µg/mL in *C. membranaefaciens* (URM4983) and 128µg/mL in *Kloeckera apiculata* (URM5002).

ConBr showed antifungal activity in ten isolates, *C. albicans* (URM4987), *C. guilliermondii* (URM4975), *C. membranaefaciens* (URM4983), *C. parapsilosis* (URM4984), *C. shehatae* (URM4978), *C. tropicalis* (URM6084), *C. tropicalis* (URM6089), *C. tropica*lis (URM4989), *K.apiculata* (URM5002), *T. cutaneum* (URM4973) with geometric mean concentrations ranging from 2-256µg/mL. (Table 2).

Table 2. Determination of minimum inhibitory concentration of lectins and DRL ConBr in yeast strains isolated from vaginal secretions.

Yeasts	DRL				Conbr			
	*MICs	*MIC_{50}	*MIC_{100}	*GMMIC	*MICs	*MIC_{50}	*MIC_{100}	*GMMIC
species	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)
URM 4987	R	R	R	R	8	16	ND	11,3
URM 4990	R	R	R	R	R	R	R	R
URM 4986	R	R	R	R	R	R	R	R
URM 4979	R	R	R	R	8	8	ND	8
URM 4985	R	R	R	R	R	R	R	R
URM 4975	128	128	ND	128	8	16	ND	11,3
URM 4976	R	R	R	R	R	R	R	R
URM 4983	64	64	ND	64	2	8	ND	4
URM 4982	R	R	R	R	8	16	ND	11,3
URM 4984	R	R	R	R	R	R	R	R
URM 4970	R	R	R	R	R	R	R	R
URM 4972	R	R	R	R	R	R	R	R
URM 4974	R	R	R	R	R	R	R	R
URM 6088	R	R	R	R	R	R	R	R
URM 4978	4	4	ND	4	2	2	ND	2
URM 6084	R	R	R	R	R	R	R	R
URM 6090	R	R	R	R	R	R	R	R
URM 6089	R	R	R	R	R	R	R	R
URM 4989	R	R	R	R	8	16	ND	11,3
URM 4981	R	R	R	R	R	R	R	R
URM 4980	R	R	R	R	R	R	R	R
URM 5002	64	256	ND	128	256	256	ND	256
URM 5092	R	R	R	R	R	R	R	R
URM 6085	R	R	R	R	R	R	R	R
URM 6086	R	R	R	R	R	R	R	R
URM 6087	R	R	R	R	R	R	R	R
URM 4971	R	R	R	R	R	R	R	R
URM 4988	R	R	R	R	R	R	R	R
URM 6083	R	R	R	R	R	R	R	R
URM 4973	R	R	R	R	2	8	ND	4
ATCC 22019	R	R	R	R	R	R	R	R
ATCC 6258	R	R	R	R	R	R	R	R

* Average results
MIC: minimum inhibitory concentrations
MIC_{50} and MIC_{100}: minimum concentration able to inhibit 50% and 100% of samples.
GMMIC: geometric mean minimum inhibitory concentration.
ND: not determined
R: resistance

LSL showed no antifungal activity in any of the isolates, while DvioL showed antifungal activity in thirteen of the strains tested, *C. albicans* (URM4987), *C.albicans* (URM4986), *C. guilliermondii* (URM4975), *C. membranaefaciens* (URM4983), *C. obtusa* (URM4982), *C. parapsilosis* (URM4984), *C. shehatae* (URM4978), *C. tropicalis* (URM6084), *C. tropicalis* (URM6090), *C. tropicalis* (URM6089), *C. tropicalis* (URM4989), *K.apiculata* (URM5002), *R. glutinis* (URM5092), with geometric mean concentrations ranging from 8-256µg/mL (Table 3).

Table 3. Determination of minimum inhibitory concentration of lectins and LSL DvioL for yeast strains isolated from vaginal secretions.

	LSL				DvioL			
Yeasts species	*MICs (µg/mL)	*MIC_{50} (µg/mL)	*MIC_{100} (µg/mL)	*GMMIC (µg/mL)	*MICs (µg/mL)	*MIC_{50} (µg/mL)	*MIC_{100} (µg/mL)	*GMMIC (µg/mL)
URM 4987	R	R	R	R	16	16	ND	16
URM 4990	R	R	R	R	R	R	R	R
URM 4986	R	R	R	R	32	32	ND	32
URM 4979	R	R	R	R	32	32	ND	32
URM 4985	R	R	R	R	R	R	R	R
URM 4975	R	R	R	R	32	32	ND	32
URM 4976	R	R	R	R	32	32	ND	32
URM 4983	R	R	R	R	128	128	ND	128
URM 4982	R	R	R	R	32	32	ND	32
URM 4984	R	R	R	R	R	R	R	R
URM 4970	R	R	R	R	R	R	R	R
URM 4972	R	R	R	R	32	64	ND	45,2
URM 4974	R	R	R	R	R	R	R	R
URM 6088	R	R	R	R	R	R	R	R
URM 4978	R	R	R	R	8	8	ND	8
URM 6084	R	R	R	R	R	R	R	R
URM 6090	R	R	R	R	32	32	ND	32
URM 6089	R	R	R	R	R	R	R	R
URM 4989	R	R	R	R	8	8	ND	8
URM 4981	R	R	R	R	R	R	R	R
URM 4980	R	R	R	R	R	R	R	R
URM 5002	R	R	R	R	16	256	ND	64
URM 5092	R	R	R	R	256	256	ND	256
URM 6085	R	R	R	R	R	R	R	R
URM 6086	R	R	R	R	R	R	R	R
URM 6087	R	R	R	R	R	R	R	R
URM 4971	R	R	R	R	R	R	R	R
URM 4988	R	R	R	R	R	R	R	R
URM 6083	R	R	R	R	R	R	R	R
URM 4973	R	R	R	R	R	R	R	R
ATCC 22019	R	R	R	R	R	R	R	R
ATCC 6258	R	R	R	R	R	R	R	R

* Average results
MIC: minimum inhibitory concentrations
MIC_{50} and MIC_{100}: minimum concentration able to inhibit 50% and 100% of samples.
GMMIC: geometric mean minimum inhibitory concentration.
ND: not determined
R: resistance

Four of the isolates, *C. guilliermondii* (URM4975), *C. shehatae* (URM4978), *C. membranaefaciens* (URM4983) and *K. apiculata* (URM5002), showed sensitivity to the three lectins with antifungal activity, DRL, and ConBr DvioL.

In addition to the strains isolated from vaginal discharge, were included two control organisms, *C. parapsilosis* (ATCC22019) and *C. krusei* (ATCC6258), both were resistant to concentrations of lectins tested. According to the p-values of Fisher's exact test ($p < 0.05$), with no differences between species in relation to sensitivity to the lectins tested, except ConBr, where the resistance was observed only in samples of *Candida* and *Rhodotourula*.

DISCUSSION

There are reports of other lectins that can bind to glucose, mannose and N-acetylglucosamine with antifungal activity. Among these, the lectin TEL has promoted 57% inhibition of growth of *Sacchromyces cerevisiae*, *Fusarium moniliforme* and *Colletotrichum lindemuthianum* in the concentration of 280μg/mL (18) and lectin AML (glucose/mannose) that caused 41% inhibition of growth of *F. solani*, *F. oxysporum* and *Colletotrichum musae* in the concentration of 100μg/mL (16). Trindade et al. (35) found the antifungal activity of lectins "jackin"and "frutackin" (N-acetylglucosamine) to the 2.25mg/mL *S. cerevisiae* and *Fusarium moniliforme*.

The antifungal activity of lectins has been detected in concentrations ranging from the 100μg/mL 10.58mg/mL for different species of fungi. There are reports on antifungal activity of lectins against yeasts as *Sacchromyces cerevisiae* (17, 35, 36).

Lectins from legumes without antifungal activity and binding specificity to glucose and mannose have also been reported by other authors, such as the lectin pea *Pisum sativum* var. *macrocarpon* (42), Chinese chestnut *Castanea mollisima* (30) and bean *Canavalia gladiata* (37).

Inhibition of fungal growth by the action of lectins, appears to be due to inhibition of spore germination as well as the growth of the mycelium (21). The exact mechanism of action has not yet been elucidated, but there seems to be alteration in the fungal cell wall due to changes in the synthesis of chitin, a deficiency in cell wall deposition. Lectins that bind to chitin showed significant antifungal effect, however, the presence or absence of this change is dependent on the combination of the fungus with the lectin (21, 33).

The antifungal activity of lectins may vary depending on the species examined. Studies have not found the antifungal activity AMML lectin with binding affinity for galactose and its derivatives in relation to *Rhizoctonia solani* and *Mycospharella arachidicola* in concentration up 200μg/mL (40). Did not observe this activity to SCL lectin with binding affinity for N-acetylgalactosamine, for *S. cerevisiae*, *C. albicans*, *Penicillium diditatum* until the concentration of 1mg/ml. However, these authors found the antifungal activity of those lectins for other species of fungi (13).

Lectins DRL ConBr and DvioL have antifungal activity, against yeasts isolated from vaginal secretion, thus forming a promising field for development of new therapeutic strategies for vaginal yeast infections and the consequent promotion of women's health.

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