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ELLSA based profiling of surface glycosylation in microorganisms reveals that ß-glucan rich yeasts' surfaces are selectively recognized with recombinant banana lectin

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Abstract

The surface of microorganisms is covered with polysaccharide structures which are in immediate contact with receptor structures on host's cells and antibodies. The interaction between microorganisms and their host is dependent on surface glycosylation and in this study we have tested the interaction of plant lectins with different microorganisms. Enzyme--linked lectin sorbent assay - ELLSA was used to test the binding of recombinant *Musa acuminata* lectin - BL to 27 selected microorganisms and 7 other lectins were used for comparison: Soy bean agglutinin - SBA, *Lens culinaris* lectin - LCA, Wheat germ agglutinin - WGA, RCA_{120} - *Ricinus communis* agglutinin, Con A - from *Canavalia ensiformis*, *Sambucus nigra* agglutinin - SNA I and *Maackia amurensis* agglutinin - MAA. The goal was to define the microorganisms' surface glycosylation by means of interaction with the selected plant lectins and to make a comparison with BL. Among the tested lectins most selective binding was observed for RCA_{120} which preferentially bound *Lactobacillus casei* DG. Recombinant banana lectin showed specific binding to all of the tested fungal species. The binding of BL to *Candida albicans* was further tested with fluorescence microscopy and flow cytometry and it was concluded that this lectin can differentiate ß-glucan rich surfaces. The binding of BL to *S. boulardii* could be inhibited with ß-glucan from yeast with IC_{50} 1.81 μg mL^{-1} and to *P. roqueforti* with 1.10 μg mL^{-1}. This unique specificity of BL could be exploited for screening purposes and potentially for the detection of ß-glucan in solutions.

Keywords

Microorganisms
Glycosylation
Banana lectin
Fungi
ELLSA

Introduction

Microorganisms have evolved a complex network of physiological adaptations that allow them to persist in many challenging environments such as the host organism. The surface of microorganisms is covered with carbohydrates, either in the form of outer layer glycoproteins, capsular polysaccharides and/or lipopolysaccharides [1]. The protruding sugar moieties can influence symbiosis and pathogenesis through cell-cell interactions, as well as recognition by the immune system, with emphasis on immune stimulation or evasion. As such, polysaccharides play important roles in colonization, survival and infection. Carbohydrate surfaces of microorganisms were acknowledged as important immunological determinants as early as the 1920s, but were then somewhat abandoned with the availability of antibiotics. In recent decades with the emergence of antibiotic resistance it is again becoming the focus of investigations [1].

Conventional methods for the analysis of glycan structures rely on HPLC based separations, capillary electrophoresis, mass spectrometry, nuclear magnetic resonance, thin layer chromatography and chemical analysis. Enrichment methods and labeling are used to improve the sensitivity of detection. Enzymatic and chemical fragmentation techniques are often necessary, because there is a requirement for prior detachment of glycans from their core proteins or molecular scaffolds [2].

During the last two decades, many different glycan arrays have been developed in order to decode the glycome, so that we could obtain insight into the roles of plant, animal and microbial glycan structures and lectin specificities. Much work has been done on the use of lectins for identifying bacterial surface carbohydrates in their native conformation, specifically for designing lectin microarrays [3, 4, 5, 6]. Lectins are carbohydrate-binding proteins or glycoproteins that bind carbohydrates without the enzymatic activity within the sugar binding site. They are generally known to have relatively low affinities, in the range of $K_D = 10^{-3}–10^{-7}$ M, but the possibility of increased avidity exists in this case because of the multimeric antigen structure and the ability of lectin molecules to form dimers and oligomers. The colonization capacity of microorganism is also largely dependent on the presence of microbial adhesins, which are lectins as well. The agglutination activity of lectin molecules can often be inhibited by simple monosaccharides but sometimes oligo/polysaccharides are required for inhibition.

Banana lectin (BL) was isolated from *Musa paradisiaca* by Koshte *et al*. [7].

The recombinant lectin which is the object of this study was produced by Gavrovic-Jankulovic *et al*. [8]. In common with other glucose–mannose-specific lectins, BL binds to α-glucosyl and α-mannosyl terminal non-reducing units [9] as well as to the branched mannose-containing oligosaccharides which are components of the so-called core region of N-linked glycoproteins, as well as to ß-1,3-linked glucosyl oligosaccharides and gentiobiosyl groups [10, 11, 12]. BL had been well characterized and its crystal structure had been revealed [13, 14] and to the best of our knowledge so far it had not been tested in a glycan array.

The goal of this work was to design an in house enzyme--linked lectin sorbent assay (ELLSA) using whole microbial cells as probes and use it for detecting lectin-sugar interactions. Further, the interaction of banana lectin with 27 selected microorganisms (upper respiratory tract pathogens, opportunistic pathogens and probiotic bacteria) was analyzed and compared with 7 widely used plant lectins in this newly developed assay. With the ultimate goal to test whether some selectivity in banana lectin binding exists.

Materials and methods

Microbial strains, growth conditions

Microbial strains used in this study are listed in Table 1. *Lactobacillus* species were propagated in MRS broth (Torlak, Serbia) at 37 °C, 5% CO_2, *Streptococcus* species were grown in BHI broth (BD, USA), while other bacterial species were grown in Nutritious broth (Torlak) at aerobic conditions at 37 °C. Fungal species were grown in Sabouraud dextrose broth (SDB) (Torlak) in aerobic conditions at 30–35 °C. Incubation period for bacterial strains was 16-24 h, and for fungal cells 24-48 h. *Aspergillus brasiliiensis* was grown at 25 °C for 72 h.

Table 1

Microorganism used in the study

	Microorganism	Phylum	Genus
1	*Lactobacillus reuteri* DSM 17938	Firmicutes G+	Lactobacillus
2	*Lactobacillus plantarum* WCFS1		
3	*Lactobacillus rhamnosus* LA68		

*CI- clinical isolate

	Microorganism	Phylum	Genus
4	*Lactobacillus rhamnosus* LB64		
5	*Lactobacillus rhamnosus* LGG		
6	*Lactobacillus helveticus* LAFTI L10		
7	*Lactobacillus acidophilus* ViVag		
8	*Lactobacillus casei* DG		
9	*Streptococcus pyogenes* ATCC 19615		
10	*Streptococcus agalactiae* ATCC 13813		
11	*Streptococcus sp.* CI β-hemolytic group B		Streptococcus
12	*Streptococcus sp.* CI β-hemolytic group A		
13	*Enterococcus faecalis* CI		Enterococcus
14	*Staphylococcus aureus* CI		Staphylococcus
15	*Proteus mirabilis* CI		Proteus
16	*Klebsiella pneumoniae* ATCC 13883		Klebsiella
17	*Pseudomonas aeruginosa* ATCC 27853	Proteobacteria G-	Pseudomonas
18	*Proteus hauseri* ATCC 13315		Proteus
19	*Shigella flexneri* ATCC 12022		Shigella
20	*Escherichia coli* ATCC 25922		Escherichia
21	*Candida albicans* ATCC 10259		Candida
22	*Candida albicans* ATCC 10231		
23	*Saccharomyces boulardii*		Saccharomyces
24	*Saccharomyces cerevisiae* ATCC 9763	Ascomycota	
25	*Aspergillus brasili~~i~~ensis* ATCC16404		Aspergillus
26	*Penicillium roqueforti* E		Penicillium
27	*Cryptococcus neoformans* 79	Basidiomycota	Cryptococcus

*CI- clinical isolate

Whole microbial cells plate coating procedure

The procedure for coating the plates with microorganisms was as previously

described [15]. Overnight cultures were centrifuged (2000×g, 20 min, 4 °C for bacterial species and 2000×g, 5 min, 4 °C for fungal species), washed with phosphate buffered saline (PBS) centrifuged as described and diluted in PBS to optical density of 0.1 at 610 nm in a final volume of 200 µL in 96 well plate. Additionally, microorganisms were counted using a hemocytometer chamber and bacterial counts mounted to $0.5–1 \times 10^8$ mL^{-1}, whereas fungal counts were $0.5–1 \times 10^7$ mL^{-1}, due to the larger size. Only *Aspergillus brasiliensis* was not treated this way, as it was not used in ELLSA.

The suspension of microorganisms was added in a volume of 100 µL per well to MaxiSorp ELISA plates (Nunc, ThermoFisher Scientific, Denmark), the plates were centrifuged (1000×g, 20 min, 24 °C) and supernatant was removed. The plates were dried at 50 °C for 2 h, and stored for no longer than 10 days.

Lectin biotinylation and dot blotting

Maackia amurensis agglutinin - MAA, Soy bean agglutinin - SBA, *Lens culinaris* lectin - LCA, Wheat germ agglutinin - WGA, RCA_{120} - *Ricinus communis* agglutinin, *Canavalia ensiformis* lectin - Con A and *Sambucus nigra* (elderberry) lectin – SNA I; were purchased from Sigma Aldrich (St. Louis, MO, USA). BL - *Musa acuminata* (banana) recombinant lectin was purified from *E. coli* cells according to previously developed procedure [8]. Lectin specificity is given in Table 2.

Table 2

Lectins' specificity

Lectin abbreviation	Source	Affinity	Ref
WGA	*Triticum vulgare*	GlcNAcß(1,4)GlcNAcß(1,4)GlcNAc> GlcNAcß(1,4)GlcNAc > GlcNAc>> Neu5Ac> > GalNAc	[16]
LCA	*Lens culinaris*	methyl-α-Man, Man, Glc	[17]
SBA	*Glycine max*	GalNAcα(1,3)Gal > GalNAc, Gal	[18]
RCA_{120}	*Ricinus communis*	terminal β-D-Gal	[19]
GlcNAc - N-acetyl glucosamine, GalNAc - N-acetyl galactosamine; Neu5Ac - sialic acid, Glc - glucose, Gal – galactose, Man -mannose.			

Lectin abbreviation	Source	Affinity	Ref
BL	*Musa acuminata*	Man, Glc, internal 3-O-α-d-glc, reducing Glc of ß-1,3-linked glucosyl oligosaccharides and ß 1,6-linked glucosyl end groups	[9, 11]
MAA	*Maackia amurensis*	Neu5Acα(2,3)Galß(1,4)GlcNAc/Glc	[20]
SNA I	*Sambucus nigra*	Neu5Acα(2,6)Gal, Neu5Acα(2,6)GalNAc	[21]
Con A	*Canavalia ensiformis*	3,6-di-O-(α-Man)αMan > Manα (1,3)Man > methyl-α-Man > Man, Glc	[22]
GlcNAc - N-acetyl glucosamine, GalNAc - N-acetyl galactosamine; Neu5Ac - sialic acid, Glc - glucose, Gal – galactose, Man -mannose.			

Briefly, lectins were dissolved in PBS, and dialyzed against 0.1 M sodium carbonate buffer pH 9.5 overnight with continuous dialysis apparatus. (+)-Biotin N-hydroxysuccinimide ester was dissolved in DMSO to the concentration of 22 mg mL^{-1} and added to the lectin solution to the 10% *V*/V. The solutions were incubated at 37 °C with constant shaking for 3 h, and were subsequently dialyzed overnight against PBS. Biotinylated lectins were analyzed with Tris-Tricine electrophoresis (8% running, 5% stacking gel). Subsequently biotinylated lectins were stored in 50% glycerol, with 0.1% sodium azide at −20 °C. Lectin concentration after biotinylation was assessed with Lowry protein concentration assay [23].

In order to assess biotinylation efficiency and to make an estimation of the appropriate working dilution dot blotting was used. After making appropriate dilutions 2 µL of solution was applied for each lectin concentration to a nitrocellulose membrane. After drying, the membrane was hydrated, blocked with 2% BSA/PBS for 30 min, and Extravidin-Alkaline Phosphatase was added and incubated for 30 min. Membrane was washed 3 times with PBS containing 0.05% Tween 20 (TPBS) and once with PBS. After washing 5-Bromo-4-chloro-3-indolyl phosphate along with Nitro-blue tetrazolium chloride was used as a substrate.

Lectin binding in ELLSA (enzyme--linked ~~L~~lectin sorbent assay)

The plates coated with microorganisms were blocked with 1% bovine serum albumin (BSA)/PBS 200 µL per well, at room temperature (RT) for 1 h. The

plates were washed 3 times with 200 µL TPBS per well and once with PBS, 200 µL per well. After the washing, for titration purposes, appropriate dilutions of lectins in PBS were added, 100 µL per well and incubated for 1 h. For typing purposes lectin dilutions used were as follows: Lectin concentrations selected for further analysis were, for BL 1 µg mL^{-1}, MAA 12 µg mL^{-1}, LCA 1.2 µg mL^{-1}, WGA 1.4 µg mL^{-1}, SBA 1.4 µg mL^{-1}, RCA_{120} 2.3 µg mL^{-1}, Con A 0.7 µg mL^{-1}, SNA I 1.5 µg mL^{-1}.

After the washing procedure same as described above, 100 µL of Extravidin-Alkaline Phosphatase (Sigma Aldrich) was added per well and incubated for 30 min at RT. *p*-Nitrophenyl Phosphate (Sigma Aldrich) was used as substrate at 1 mg mL^{-1} in alkaline phosphatase buffer, 100 µL per well. The absorbance was read at 405 nm with reference wavelength at 620 nm.

Each sample was measured in duplicate and intra-assay coefficients of variation were below or equal to 10% for all the bacterial ELLSAs performed.

Inhibition of lectin binding in ELLSA was done with lectins preincubated with different saccharides in order to test if binding inhibition occurs.

Fluorescence microscopy and flow cytometry of BL binding to ~~C. albicans~~*C. albicans* ATCC 10259 and ~~A. brasili~~*A. brasiliiensis*~~ensis~~ ATCC16404

Overnight culture of fungal cells was centrifuged 1000×g, 2 min, washed once with PBS and diluted in PBS containing 0.01% NaN_3. A total of 100 µL (OD_{620nm} = 0.5) was used. BL-biotin was added at an amount of 2 µg, mixed and incubated 15 min at 4 °C. To the control sample no BL-B was added. After washing and centrifugation twice with PBS (3 mL) Streptavidin-FITC 1 µg (Biolegend, San Diego, CA) was added, incubated for 15 min at 4 °C. After washing and centrifugation twice with PBS (3 mL) the signal was visualized with fluorescent microscopy on Olympus BH-2 (Olympus Optical Co. Ltd., Tokyo, Japan) equipped with exciter filter BG 12 and barrier filter Y495, Color View III digital camera (Olympus Soft Imaging Solutions GmbH, Münster, Germany) and AnalySIS FIVE software (Olympus Soft Imaging Solutions). For flow cytometric detection, the procedure was the same, with varying concentration of BL from 0.15–2.4 µg and the signal was analyzed on FACSVerse (Becton Dickinson, Mountain View, CA, USA). *A. brasili~~i~~ensis* could not be analyzed in this way as it could not be centrifuged, so it was stained by transferring the mycelium with tweezers into different solutions. A

negative control was obtained by adding 100 mM glucose to BL-B and staining with Streptavidin-FITC, upon which it was visualized under the fluorescent microscope.

ß-glucan ELLSA

The procedure was adopted from [24] with brief modifications: 2.5 mg mL^{-1} polysaccharide suspension of ß-glucan from *S. cerevisiae* (Sigma) was used for coating, the plate was blocked with 1% BSA/PBS, and washing was done with (TPBS).

Visualization and data analysis

All graphs were produced in Origin Lab 8.0 and GraphPad Prism 5. For calculation of IC_{50} values GraphPad Prism 5 was used.

Results

Lectin binding analysis and endpoint titration

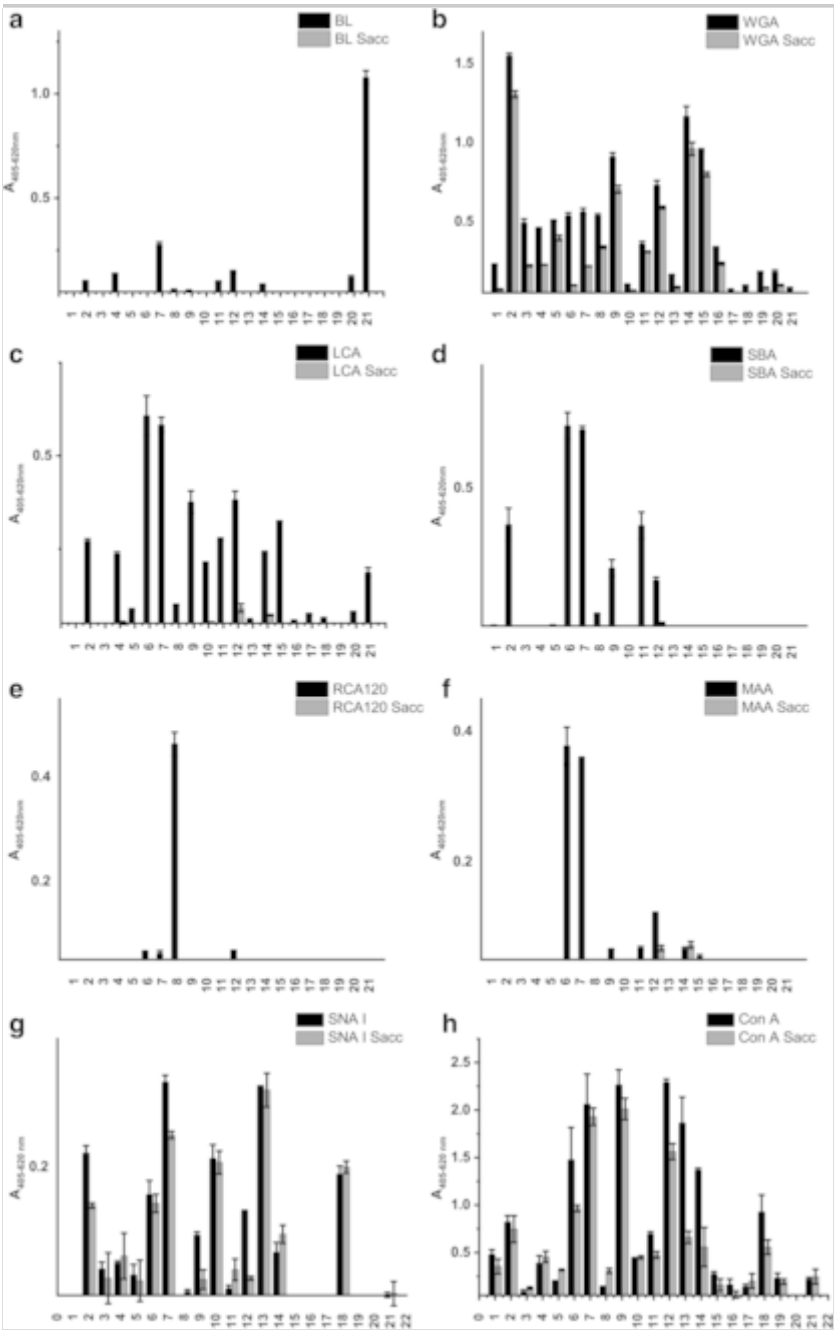
The biotinylated lectin preparations had protein concentrations in the range 1–3 mg mL^{-1}, and dot blotting analysis have shown that the biotinylation efficiency was not equal for all the lectins, Supplement 1. The biotinylation of MAA was less successful, and it was used in higher concentrations for further analysis.

In order to assess the optimal lectin concentrations to be used in the assay a titration experiment was performed with a mixture of equal amounts of microorganisms (Supplement 2). The reactivity of BL, Con A and WGA was markedly higher than that of other lectins used. MAA reactivity could not be detected in this way, but was still tested at a lower dilution.

The binding of individual lectins for individual microorganisms is shown in Fig. 1; A-H. The values presented were confirmed by three independent experiments. WGA and Con A bound to the majority of microorganisms tested (Fig. 1B, H) and were hence most non-specific for the bacterial strains used in this study. LCA, SBA and SNA I also displayed less selectivity (Fig. 1C, D and G) whereas other tested lectins showed more selectivity, detecting saccharide structures only on certain microorganisms. BL selectively bound *C. albicans* ATCC 10259 (Fig. 1A), RCA_{120} to *L. casei* DG (Fig. 1E) and MAA to *L. helveticus* LAFTI and *L. acidophilus* ViVag (Fig. 1F).

Fig. 1

The binding of biotinylated lectins to microorganisms in ELLSA. Lectin molecules used: BL, WGA, LCA, SBA, RCA, MAA, SNA I and Con A. Sacc – 0.5 M monosaccharide mixture (glucose, mannose, galactose). Numbers mark the following microorganisms: 1. *L. reuteri* DSM 17938, 2. *L. plantarum* WCFS1, 3. *L. rhamnosus* LA68, 4. *L. rhamnosus* LB64, 5. *L. rhamnosus* LGG, 6. *L. helveticus* LAFTI L10, 7. *L. acidophilus* ViVag, 8. *Lactobacillus casei* DG, 9. *S. pyogenes* ATCC 19615; 10. *S. agalactiae* ATCC 13813; 11. *Streptococcus* sp. CI β-hemolytic group B; 12. *Streptococcus* sp. CI β-hemolytic group A; 13. *E. faecalis* CI; 14. *S. aureus* CI; 15. *P. mirabilis* CI; 16. *E. coli* CI; 17. *K. pneumoniae* ATCC 13883; 18. *P. aeruginosa* ATCC 27853; 19. *P. hauseri* ATCC 13315; 20. *S. flexneri* ATCC 12022; 21. *C. albicans* ATCC 10259

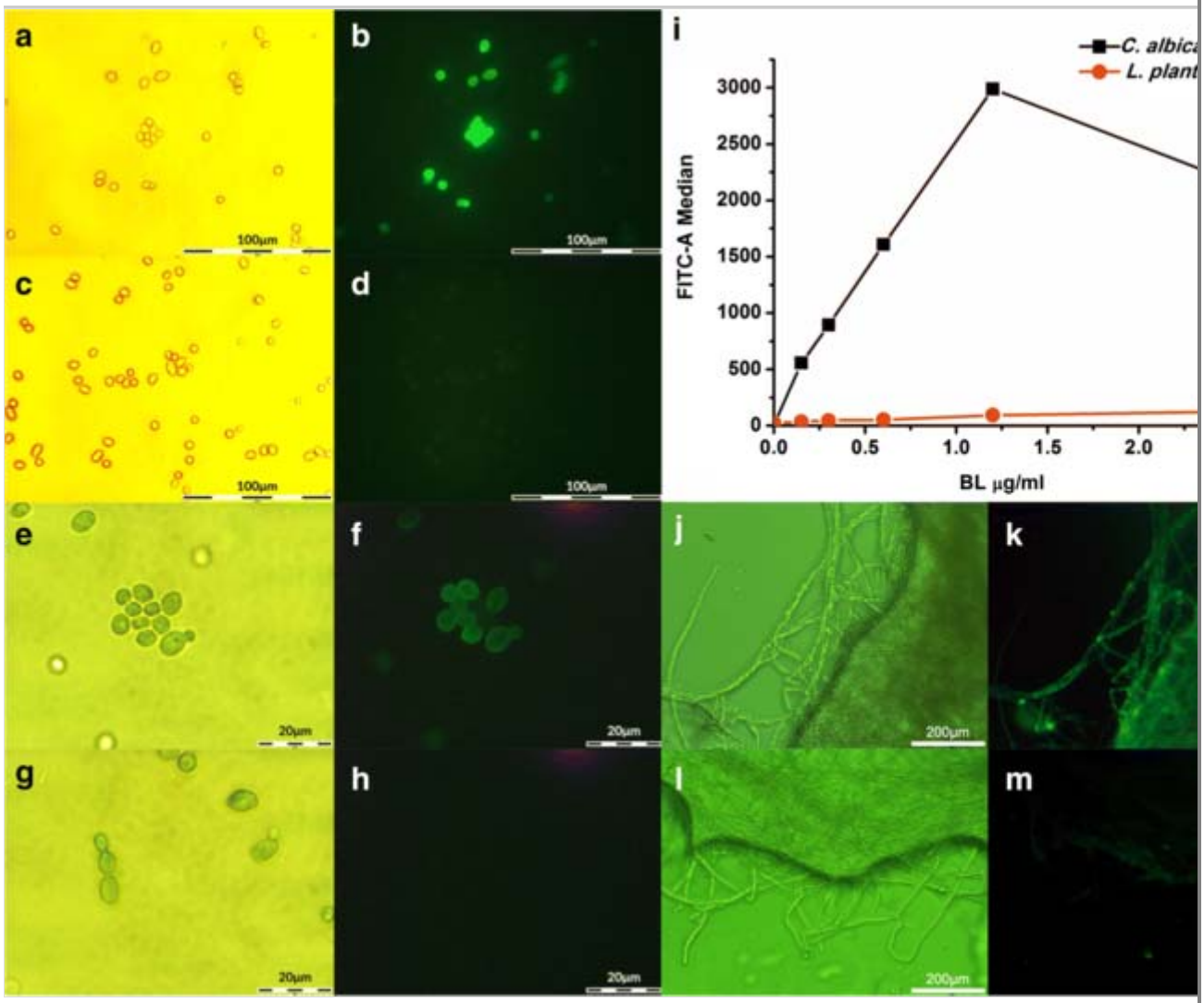


The binding of BL to *C. albicans* was confirmed with fluorescent microscopy (Fig. 2B and F) and flow cytometry, where a plateau was reached at the concentration above 1 μg ml^{-1} (Fig. 2I). Additionally, the sugar mediated binding of BL to surface of one other yeast species *A. brasili~~i~~ensis* was confirmed with fluorescent microscopy (Fig. 2J-M).

Fig. 2

Fluorescent microscopic detection of BL binding to *C. albicans* ATCC 10259 and *A. brasiliiensis* ATCC16404. A, E - light and B, F- fluorescent image of *C. albicans* stained with BL-biotin and visualized with streptavidin-FITC, C, G - light and D, H - fluorescent image of *C. albicans* stained with streptavidin-FITC (control). A, B, C, D – 10 x magnification; E, F, G, H – 40 x magnification. I-

Flow cytometric detection of BL binding to *C. albicans*; *L. plantarum* WCFS1 was used as negative control. ~~K, L~~J, K – light and fluorescent image of *A. brasili~~i~~ensis* stained with BL-biotin without added glucose and ~~M, N~~L, M - light and fluorescent image with added glucose; 10 x magnification

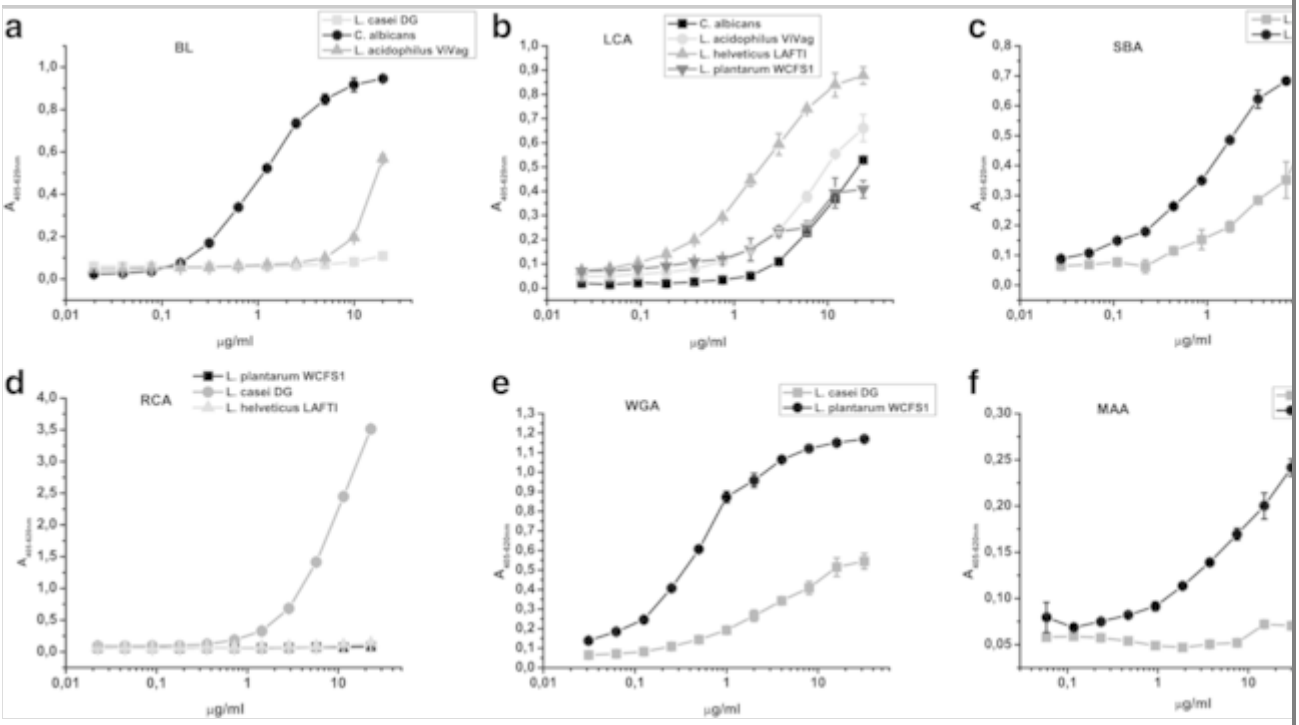


Next we performed ELLSA titration experiments in order to see if dose dependent saturation occurred. In Fig. 3 it is shown that saturation was achieved for BL binding to *C. albicans* (Fig. 3A), or for LCA and SBA binding to LAFTI and WCFS1 (Fig. 3B, C) and for WGA binding to WCFS1 (Fig. 3E). The endpoint was variable depending on the lectin molecule used, and was in the range of 0.3 µg mL^{-1} for BL - *C. albicans* interaction, 0.2 µg mL^{-1} for LCA – LAFTI interaction, 0.1 µg mL^{-1} for SBA – LAFTI interaction, 1 µg mL^{-1} for RCA_{120} *L. casei* DG interaction, 0.05 µg mL^{-1} for WGA – *L. plantarum* interaction and 1 µg mL^{-1} for MAA – LAFTI interaction. The absence of saturation for MAA and RCA_{120} reactivity was also evident in the higher value of endpoint titration. This raises the question

of the specificity/affinity of these interactions. The interaction was inhibited with a monosaccharide mixture and was hence a lectin-sugar interaction. However, the absence of saturation might imply that the bacteria do not express the optimal lectin ligand, or that the concentration of the ligand on the bacteria was not at zone of equivalence.

Fig. 3

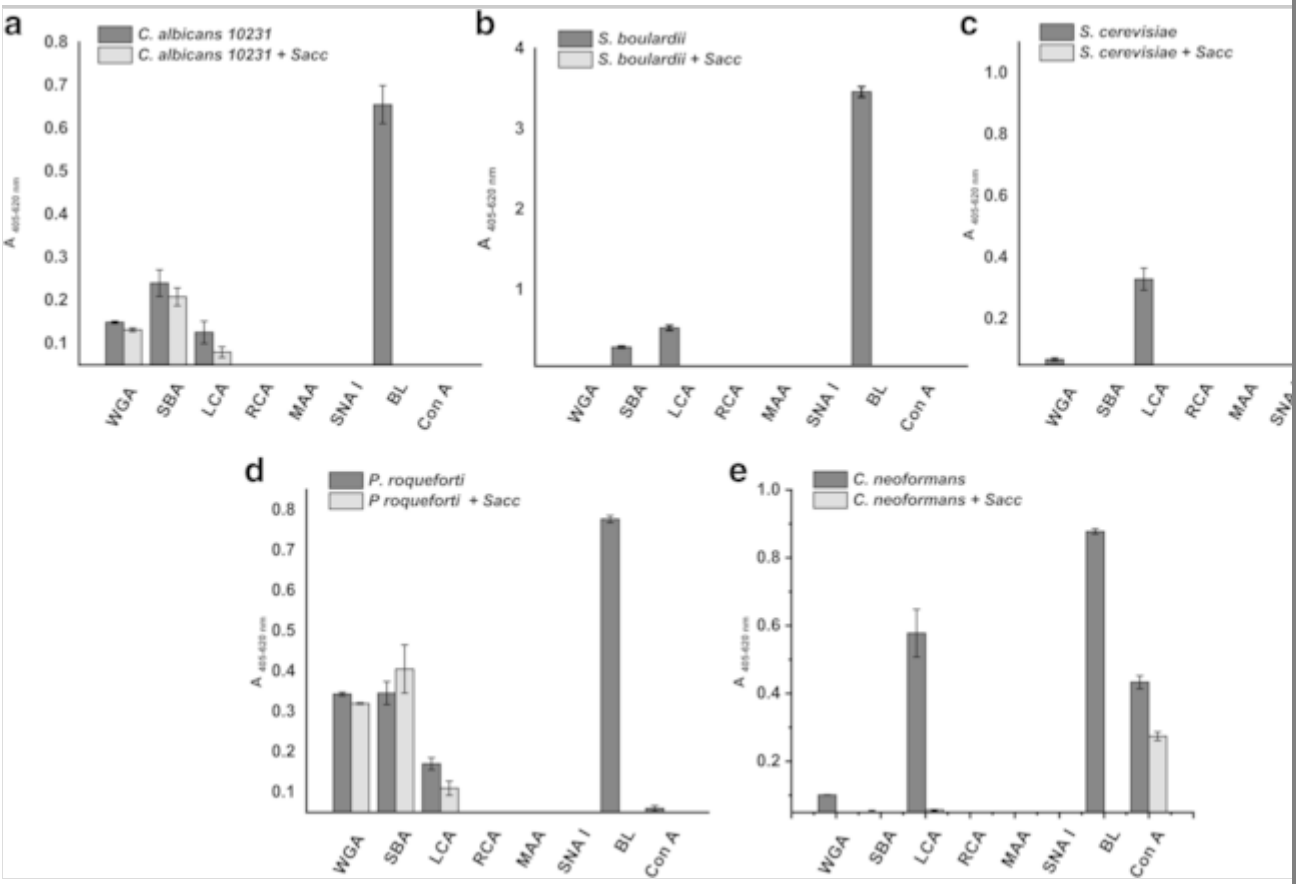
Titration of lectin reactivity with microorganisms. Whole microorganism coated plates were probed with different dilutions of: A) BL; B) LCA; C) SBA; D) $RCA_{120;}$ E) WGA; F) MAA



The selective binding of banana lectin to fungal β-D-glucan which was observed for *C. albicans* was further tested with other fungal species (Fig. 4). BL bound to all of the tested fungal species, without exception and the binding was higher compared to any of the other tested lectins.

Fig. 4

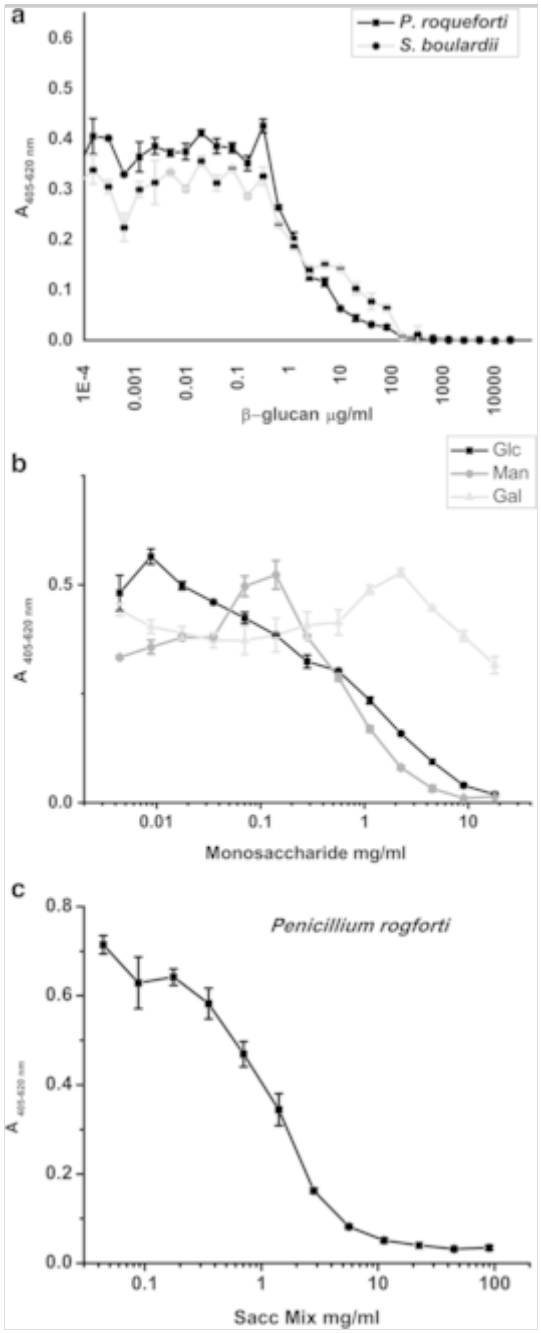
The binding of plant lectins to selected fungi. A) *C. albicans* ATCC 10231, B) *S. boiulardii*, C) *Saccharomyces cerevisiae* ATCC 9763, D) *Penicillium roqueforti* E, E) *Cryptococcus neoformans* 79. Wheat germ agglutinin – WGA; Soy bean agglutinin – SBA; *Lens culinaris* lectin - LCA; RCA_{120} - *Ricinus communis* agglutinin; *Maackia amurensis* agglutinin – MAA, *Sambucus nigra* agglutinin – SNA I, *Musa acuminata* (banana) recombinant lectin – BL and Concanavali A - Con A



The inhibition of banana lectin binding to fungi was tested with ß-glucan (Fig. 5A). IC_{50} of ß-glucan from *S. cerevisiae* for the binding of BL to *S. boulardii* was 1.81 µg mL^{-1} (95% confidence interval: 0.82–3.97 µg mL^{-1}) and to *P. roqueforti* - 1.10 µg mL^{-1} (0.62 to 1.95 µg mL^{-1}).

Fig. 5

A) Inhibition of banana lectin binding to two yeast species *P. roqueforti* and *S. boulardii* with ß-glucan from *S. cerevisiae*. Inhibition of banana lectin binding to *Penicillium roqueforti* with B) individual monosaccharides and C) monosaccharide mixture (Glc, Man, Gal)

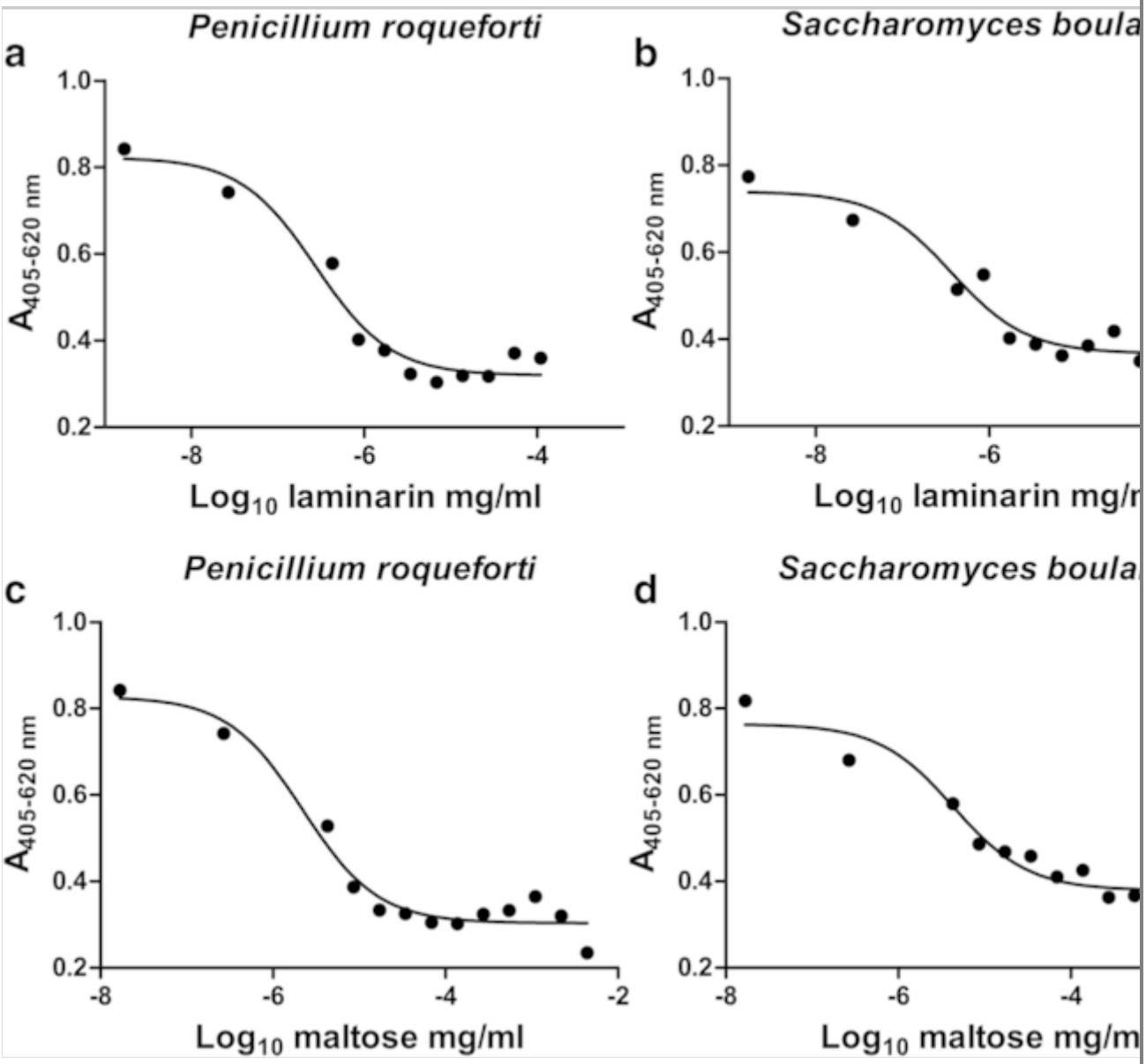


The influence of individual monosaccharides on binding inhibition of banana lectin to *P. roqueforti* was also tested Fig. 5B. IC_{50} for glucose was 0.77 mg mL^{-1} (0.23–2.58 mg mL^{-1}), for mannose 1.27 mg mL^{-1} (0.32–5.07 mg mL^{-1}), whereas galactose did not inhibit this interaction.

Additionally laminarin and D-maltose were also evaluated as inhibitors, Fig. 6. Laminarin inhibited the binding of BL to *P. roquefori* with IC_{50} 0.26 ng mL^{-1} (0.12–0.55 ng mL^{-1}) and *S. boulardii* 0.33 ng mL^{-1} (0.14–0.77 ng mL^{-1}). D-Maltose inhibited the binding of BL to *P. roquefori* with IC_{50} 2.24 ng mL^{-1} (1.16–4.33 ng mL^{-1}) and *S. boulardii* 3.42 ng mL^{-1} (2.1–5.57 ng mL^{-1}).

Fig. 6

Inhibition of banana lectin binding to *P. roqueforti* with: A) laminarin; C) D-Maltose and to *S. boulardii* with B) laminarin; D) D-Maltose

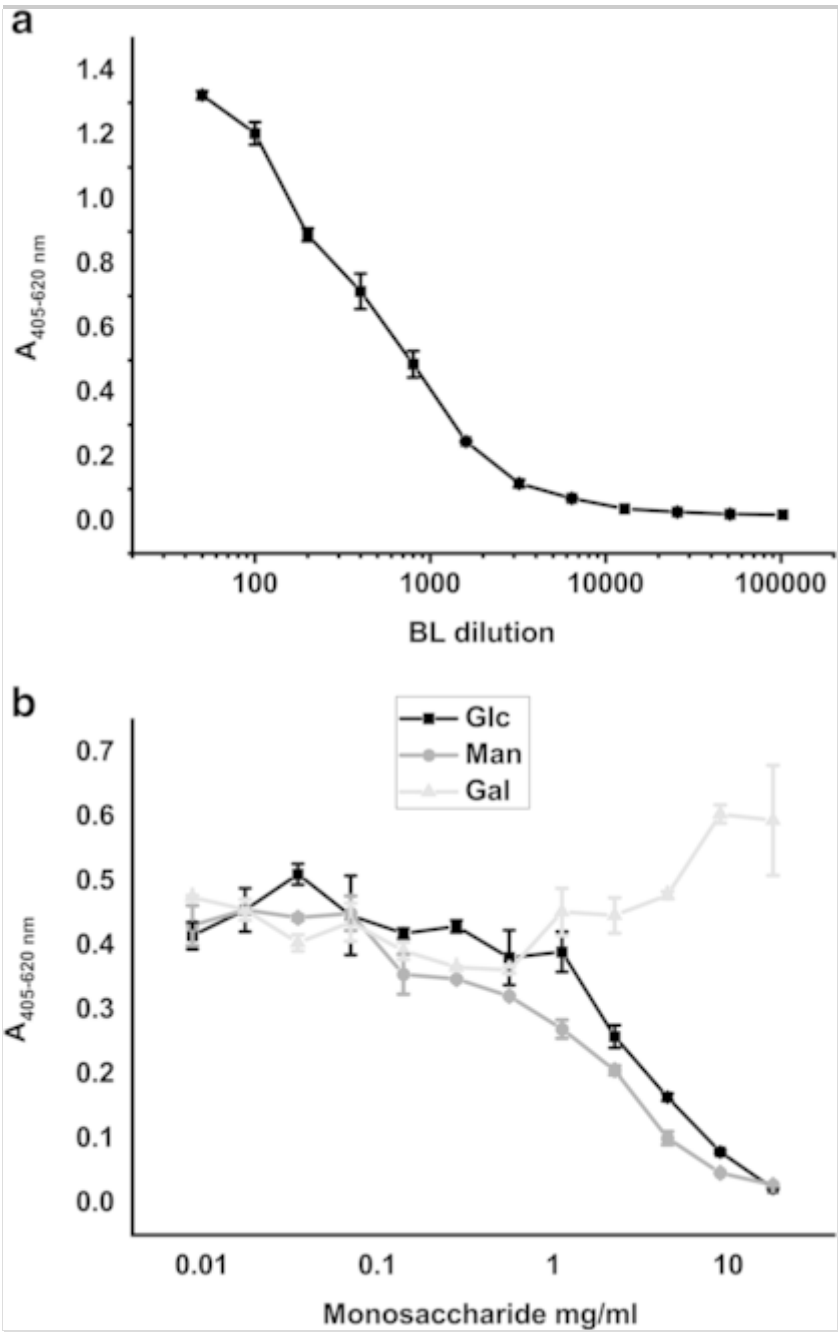


Next we tested the binding of BL directly to ß-glucan. Titration curve for the binding of BL to ß-glucan is shown in Fig. 7A. At BL concentration used for glucan detection in the microorganism coated ELLSA the 50% inhibition of BL binding to β-D-glucan was achieved with 4.33 mg mL^{-1} (2.23–8.41 mg mL^{-1}) glucose and 1.72 mg mL^{-1} (1.03–2.87 mg mL^{-1}) mannose, while galactose had no effect on binding Fig. 7B.

Fig. 7

The binding of BL to ß-glucan from *S. cerevisiae* in ELLSA. A) BL binding curve; B) The inhibition of binding of banana lectin to ß-glucan with

monosaccharides, glucose, mannose and galactose



Discussion

The interaction of lectin molecules with various microorganisms is determined by the accessible outer layer sugar moiety. Certain lectins specifically bind certain kinds of microorganism almost exclusively; such as the case of banana lectin binding to fungi detected in this study. Within the *C. albicans* inner cell wall β-(1,3)-glucans are key structural polysaccharides [25] and the outer layer of the *C. albicans* cell wall is composed of an array of heavily mannosylated proteins that are cross linked to β-(1,6)-glucans [26, 27,

28]. The outer mannan layer is thought to play an important role in concealing β-glucans from host immune detection, in particular from Dectin 1 [29, 30]. On the other hand there are endogenous lectins which recognize mannose residues of *Candida.* Such lectins are dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin DC-SIGN, also known as CD209 [31] and the mannose receptor (MR, also known as CD206) of macrophages [32]. Mannose-binding lectin (MBL, also known as MBL2) is likely another important host receptor for *C. albicans* mannans [33, 34].

In this study three mannose binding lectins were used: LCA which recognizes α-linked mannose residues, Con A which recognizes mannose in 3,6-di-O-(α-Man)αMan configuration and banana lectin (Table 2). Interestingly, both of the tested *C. albicans* strains displayed low or no binding to Con A, whereas LCA bound only to one of the strains. Whether this is a strain specific issue, or is related to the cultivation method or the ELLSA methodology is unknown and this generally differs from literature data. On the other hand the binding of both Con A and LCA was detected for *S. cerevisiae* and *C. neoformans.* BL was the third mannose binding lectin used, and it did not discriminate between different fungi.

It is clear that banana lectin is markedly different from the other two lectins and that its primary ligand is ß-glucan expressed on fungal cells. Among the tested microorganisms banana lectin binding was detected almost exclusively for the tested fungi, which implies other fungal cells rich in ß-glucans would also bind this lectin. Low reactivity with the tested bacterial cells does not exclude the reactivity with bacteria which possess surface exposed ß-glucans [35]. The affinity of natural banana lectin was previously described as peculiar for it recognizing ß-1,3-linked glucosyl oligosaccharides and ß 1,6-linked glucosyl end groups (gentiobiosyl groups) which occur in many fungal ß1,3/1,6-linked polysaccharides [9, 11].

The specific/selective binding of lectins to accessible outer layer saccharides of a microorganism is a desired phenomenon, as it could be applied in the design of specific reagents for microorganism identification. For instance, the binding of RCA_{120} to the surface of *L. casei* DG implied the existence of galactose residues on the surface of this strain. Several *L. casei* strains were evaluated by Yasuda and coworkers in a lectin microarray, and one of the strains isolated from saliva showed marked reactivity towards RCA_{120} [36] as we obtained here for the DG strain. Recently *L. casei* DG was found to have a

novel, branched hetero-exopolysaccharide with a repeat unit composed of L-rhamnose, D-galactose, and N-acetyl-D-galactosamine in a ratio of 4:1:1 [37], and therefore this microorganism can be used as a positive control for RCA_{120} binding.

We have also tested antifungal activity of high doses of banana lectin against *C. albicans* and *C. neoformans* and obtained no growth inhibition (unpublished results). There is a possibility that banana lectin could aid in displacement from mucosal surfaces but it remains to be tested.

Whole microbial cell ELLSA, presented here is inferior from the lectin microarray technology in terms of capacity, but, is relatively simple in terms of experimental methodology and it provides useful information which could be widely applied [38]. The possible applications comprise quick screening of newly isolated microbial clones for desired selective traits, in terms of surface polysaccharide composition or the screening of suspected culprits amongst microbiota in inflammatory diseases. Furthermore, this methodology could be useful in elucidating the specificities of newly discovered or mutated lectin molecules by comparison with lectins of known specificities.

Previously it was shown that banana lectin isolated from banana binds α-1,3-linked glucosyl residues [9] and ß1,6-linked glucosyl end groups (gentiobiosyl groups) as occur in many fungal ß1,3/1,6-linked polysaccharides [11] by the techniques of quantitative precipitation, hapten inhibition of precipitation, and isothermal titration calorimetry. The two mentioned and related studies represent the most detailed analysis of banana lectin specificities. Here, by using ELLSA methodology we conclude that BL is markedly different for the other 7 tested lectins and that irrespective of its ability to bind different structures, in this assay it binds specifically to surface ß-glucans.

The experimental approach presented here showed that screening with recombinantly produced banana lectin could be used for detecting the presence of ß1,3/1,6-linked polysaccharides on the surface of fungi and possibly bacteria. This was concluded from the selective binding of banana lectin to fungal ß-glucans and the absence of binding to other mannosylated microorganisms as well as from the inhibition assays. Therefore, this study could represent a valuable reference as it provides additional possibilities of the usage of recombinantly produced banana lectin, due to its specificity. Although not analyzed in this study this new insight could possibly help establish alternative ways of detection and quantification of ß-glucans in

fluids.

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Author contributions

Rajna Minic, Vesna Ilic and Marija Gavrovic-Jankulovic made substantial contributions to conception and design of the study. Material preparation and data collection was done by Luka Dragacevic and Danijela Kanazir. Data analysis and interpretation was done by all authors. The first draft of the manuscript was written by Rajna Minic and Luka Dragacevic, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animals preformed by any of the authors.

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