

Interaction of some plant lectins with poultry gastrointestinal pathogenic bacteria – an alternative to antibiotic therapy

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There were performed in vitro interactions of five plant lectins purified by affinity chromatography with seven pathogenic bacteria cultivated in liquid media. The positive results were scored by the degree of bacterial cells agglutination. There have been noticed strong agglutinations of *Salmonella typhimurium* and *Salmonella gallinarum* with the lectins isolated from pumpkin seeds, potato buds and potato tubers. *Escherichia coli* and *Pseudomonas aeruginosa* were better agglutinated by vegetable marrow and pumpkin seeds lectins and weaker by potato buds and tubers lectins.

Glutaraldehyde immobilization of the lectin isolated from potato buds resulted in an insoluble polymer suspension. The interaction of the lectin polymer suspension with the liquid cultures of *Salmonella* and *Pseudomonas* was quantified by measuring the OD (660 nm) at time 0 and after 10 minutes of standing. The decrease of the OD demonstrated that immobilized insoluble lectin polymer bound bacterial cells and the aggregates settled. The experiments were performed in five replicate each and the results proved to be statistically significant ($P < 0.01$). Gram stained slides of the sediments showed the bacterial binding to the lectin polymer. These results suggest that immobilized lectins could be included in the feed formula in order to prevent bacterial infections.

Keywords: lectin-bacteria interaction; immobilized lectin.

Introduction

Immunization strategies and antibiotics use in farm animals is confronted with a series of limitations because of the increasing concern about drug residues in the food products. Prevention of both infections and parasitosis diseases is the best approach for these diseases. Recent progress in glycobiology emphasized that numerous pathogens, from viruses and bacteria to pluricellular parasites use lectins as tools to recognize and bind to the oligosaccharides exposed by target cells and tissues, by lectin mechanisms (Imberty et. al.). On the other side, the pathogens surfaces bear a large number of oligoglucides that may be bound by specific lectins that can modulate the host infection (Naughton et. al.; Munoz-Crego et al., Schmidt et al.).

The complex interactions among pathogens and hosts and pathogens- immune system, some of them still unclear, determined an increasing fight against pathogens, especially by chemical drugs use. The huge ability of microorganisms to adapt easily to new environmental circumstances helped them to find the suitable weapons to survive, and the multiple antibiotic resistance of pathogenic bacteria became a major problem worldwide. The present study focuses on the identification of lectins able to agglutinate pathogenic bacteria in order to prevent their adhesion to the target.

Materials and methods

Bacterial strains were grown on liquid media for 24 h at 37°C (brain-heart broth for *Salmonella* and *Pseudomonas* and Luria broth for the others).

Lectins were extracted and purified by affinity chromatography on suitable supports.

Agglutination assays were done on microtitre plates using 50µL lectin solution and the same amount of bacterial suspension (Allen).

Glutaraldehyde lectin immobilization was made by mixing under mild agitation the polymerising agent to the lectin solution (3 parts lectin:1 part glutaraldehyde). After 1 h, the mixture was left at room temperature 12 h. The lectin molecules formed a nonhomogenous insoluble polymer that was separated and washed by successive centrifugations. After the last wash (when the supernatant OD at 280 nm was less than 0.01), the sediment was resuspended in PBS pH 7.2.

Bacterial suspension – immobilized lectin interaction was done by incubating at 37°C 1.5 mL bacterial suspensions with 0.5 mL lectin suspension. OD at 660 nm was measured at the first moment of reaction (t_0) and after 10 minutes of incubation (t_{10}), while the tubes were not disturbed during incubation, to allow the polymer-cells aggregates to settle. This was an adaptation after Touhami and col.

Slides stained by Gram method were done from deposits of immobilized lectin-bacterial suspensions and also from the immobilized lectin (as control) and examined using an optical microscope.

Results and discussion

The agglutination assays showed strong agglutinations of *Salmonella typhimurium* and *Salmonella gallinarum* with the lectins isolated from pumpkin seeds and potato buds and potato tubers. *Escherichia coli* and *Pseudomonas aeruginosa* were better agglutinated by vegetable marrow and pumpkin seeds lectins and weaker by potato buds and tubers lectins. The other bacterial species were also agglutinated (Table 1). In Figure 1 is presented the agglutination pattern of *Pseudomonas aeruginosa* with the lectin isolated from pumpkin seeds (*Cucurbita pepo*). It is also important to notice that all the lectins that efficiently agglutinated bacterial strains had a related carbohydrate specificity (N-acetylglucosamine and its oligomers).

Table 1 Lectins – bacteria interaction. Agglutination scored (+,-) after five minutes

	Vegetable marrow (<i>Cucurbita pepo ovifera</i>)	Pumpkin seeds (<i>Cucurbita pepo</i>)	Potato tubers (<i>Solanum tuberosum tubers</i>)	Potato buds (<i>Solanum tuberosum buds</i>)	Black radish seeds (<i>Raphanus sativus</i>)
<i>Pseudomonas aeruginosa</i>	+++	+++	+	++	+
<i>Escherichia coli</i>	+++	+++	++	++	-
<i>Salmonella typhimurium</i>	++	+++	+	+++	-
<i>Salmonella gallinarum</i>	+++	+++	+	+++	+/-
<i>Bacillus cereus</i>	-	+	++	+	-
<i>Klebsiella sp.</i>	+	++	+++	+++	+
<i>Burkholderia cepacia</i>	-	+++	++	+++	++

Glutaraldehyde immobilization of the lectin isolated from potato buds resulted in an insoluble polymer suspension. This lectin was chosen because it agglutinated all the tested bacteria and also, in previous experiments, it resisted at the proteolytic attack in the gastrointestinal tract, was absorbed and induced systemic effects like hypoglycemia in diabetic mice (Pop et al.). The interaction of the lectin polymer suspension with the liquid cultures of *Salmonella* and *Pseudomonas* was quantified by measuring the OD (660 nm) at time 0 and after 10 minutes of standing. The decrease of the OD demonstrated that immobilized insoluble lectin polymer bound bacterial cells and the aggregates settled. The experiments were performed in five replicate each and the results proved to be statistically significant ($P < 0.01$, table 2).

Table 2 Variations of OD-660 nm at the start and after 10 minutes of immobilized-lectin bacteria intercation

Sample	<i>Pseudomonas</i>			<i>Salmonella</i>		
	T ₀	T ₁₀	Δ OD	T ₀	T ₁₀	Δ OD
1	0.97	0.797	0.173	0.4	0.187	0.213
2	0.95	0.78	0.17	0.41	0.208	0.202
3	0.91	0.783	0.127	0.4	0.2	0.2
4	0.97	0.79	0.18	0.39	0.195	0.195
5	0.93	0.765	0.165	0.43	0.21	0.22
Mean	0.946	0.783	0.163*	0.406	0.2	0.206*

* $P < 0.01$

Gram stained slides of the sediments showed the bacterial binding to the lectin polymer (figure 1,2,3,4).

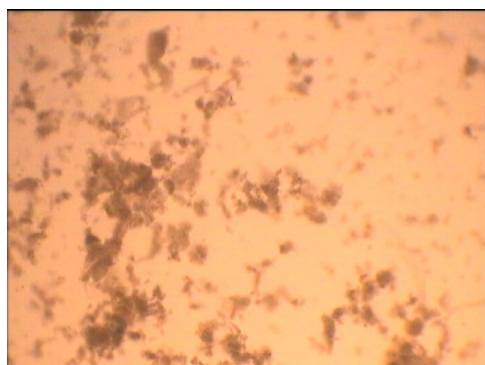


Figure 1 *Pseudomonas* agglutination by pumpkin seeds lectin



Figure 2 Gram stained slide of the immobilized lectin

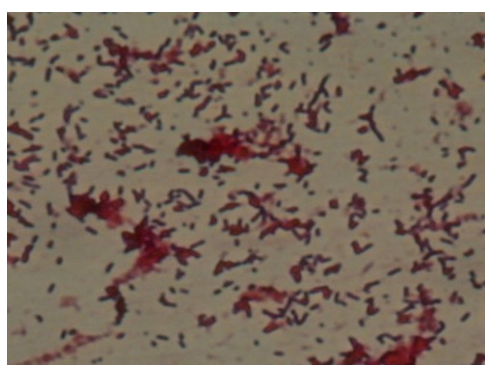


Figure 4 *Pseudomonas* binding to the immobilized lectin (Gram staining)

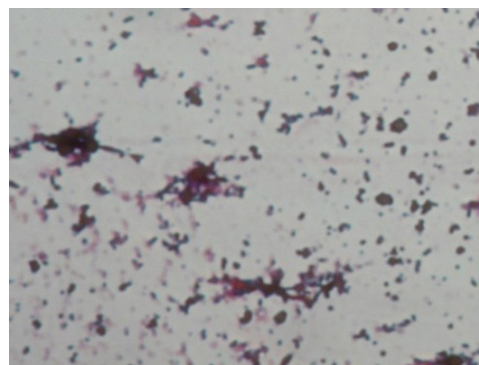


Figure 5 *Salmonella* binding to the immobilized lectin (Gram staining)

It should be pointed out that the immobilized lectin used is a glycoprotein and both *Salmonella* and *Pseudomonas* possess their own lectins. The main advantage of the immobilized lectin use is that it is not important if the lectin binds bacteria or the bacterial lectins attach them to the lectin polymer as long as they are stuck together. The immobilization of the lectin may prevent its absorption and the subsequent systemic effects.

These results suggest the possibility of plant lectins utilization for the prevention of some bacterial infections. For those bacterial strains that have an oral route of infection it may be important to test the impact of potato buds immobilized lectin *in vivo*.

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