Interaction of Some Pathogenic Bacteria with Phaseolus Vulgaris L. Seeds Lectin (as Biosensor)

Mohammed A. Jebor and Yasser H. Jalil

***Abstract*—A lectin has been isolated from phaseolus vulgaris and purified by ion exchang and gel filtration . The purified lectin was immobilized with glutaraldehyde. The in vitro interaction of free and immobilized lectin with nine pathogenic bacteria (E. coli , Bacillus , Enterobacterium , Salmonella , Gardenella , Staphylococcus ,Streptococcus , Morganella , Pseudomonas) were study . The result showing agglutination of six pathogenic bacteria (Bacillus , Enterobacterim , Salmonella , Staphylococuss , Streptococus , Pseudomonas) with free lectin. Glutaraldehyde immobilized lectin purified from Phaseolus vulgaris resulted in an insoluble polymer suspension. The interaction of lectin polymer suspension with liquid culture of pathogenic bacteria was quantified by measuring the OD (660 nm) at time zero and after ten minutes from incubation.**

The results showed strong agglutination of immobilized lectin with Bacillus, Staphylococcus, Streptococcus, and Pseudomonas more than agglutination with E. coli, Enterobacterium, Morganella and Gardenella.

***Index Terms*—Immobilization, lectin, lectin-bacteria interaction.**

I. Introduction

Lectins are defined as proteins / glycoproteins possessing at least one non-catalytic domain which binds reversibly to a specific mono- or oligosaccharide [1]. Because the specifities of lectin to bind carbohydrates and because the pathogens surfaces bear a large number of carbohydrate (oligosaccharides) so we use it in immobilized image to precipitate bacterial cells. Immobilization strategies are various depending on the purpose of immobilization, if the goal of immobilization is for purified lectin (affinity chromatography) the lectin bind non-covalently (reversibly) with carbohydrates which immobilized to matrices (covalently), and if the goal of immobilization is to use the immobilized lectin to immobilized or precipitate cells the lectin will immobilized covalently (irreversibly) to matrix as sepharose, sephadex or agarose. In this research we immobilized lectin by gluteraldehyde (covalently bond) and using it to bind with carbohydrates receptors on cell surface as antibiotics [2], can immobilized lectin on agarose to detect soluble membrane receptors , also Glycoprotein's of Sendai virus were isolated on a column of Helix pomatia lectin- Sepharose 6MB as a preparative step for the study of viral glycoprotein's and immobilized it [3].There are several strategies to immobilized protein such as Nonpolymerizing Covalent Immobilization, Cross-Linking with Bifunctional Reagents, Adsorption, Entrapment and Microencapsulation, the immobilization technique used mostly to immobilized enzyme also for cell and other protein but there is some properties or behavior must be found in the immobilized molecule, The behavior of immobilized enzymes differs from that of dissolved enzymes because of the effects of the support material, or matrix, as well as conformational changes in the enzyme that result from interactions with the support and covalent modification of amino acid residues. Properties observed to change significantly upon immobilization include specific activity, pH optimum, Km, selectivity, and stability. Physical immobilization methods, especially entrapment and encapsulation, yield less dramatic changes in an enzyme's catalytic behavior than chemical immobilization methods or adsorption. The reason is that entrapment and encapsulation result in the enzyme remaining essentially in its native conformation, in a hydrophilic environment, with no covalent modification [4]. Antibiotics are substances which, even at low concentrations, inhibit the growth and reproduction of bacteria and fungi. The treatment of infectious diseases would be inconceivable today without antibiotics. Substances that only restrict the reproduction of bacteria are described as having bacteriostatic effects (or fungistatic for fungi). If the target cells are killed, then the term bactericidal (or fungicidal) is used. Almost all antibiotics are produced by microorganisms—mainly bacteria of the genus Streptomyces and certain fungi. However, there are also synthetic antibacterial substances, such as sulfonamides and gyrase inhibitors [5]. Use in farm animals is confronted with a series of limitation because of the increasing concern about drug residues in the food products. Prevention of both infection and parasitiosis is the best approach for these diseases. From virus and bacteria to parasites use lectin tools to recognize and bind to the oligosaccharides exposed by target cell and tissue, by lectin mechanisms [5].

In bacteria, numerous surface structures bind lectins, such as peptidoglycan, teichoic acids, lipopolysaccharides, and capsular materials [6]. The complex interactions among pathogens and hosts, some of them still unclear, determination an increasing fight against pathogens, especially by chemicals drugs uses. This study focused on using lectin in both; immobilized and free image to agglutinate some pathogenic bacteria to prevent them from adhesion to the target [7].

Some type of lectin recognize specific carbohydrate (one or two) such as lectin from Helix pomatia snail which recognized N-acetyl-D-galactosamine residues , it was

Manuscript received October 10, 2012; revised February 18, 2013.

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considered this lectin would react with group C beta-hemolytic streptococci have a similar terminal aminocarbohydrate as part of their group carbohydrate [8].

But some type of lectin as conA (Concanavalia ensiformis), WGA (Weat germ aglutinine) and PHA (phytohemoagglutinie from phaseolus vulgaris) have acomplex specifities to carbohydrates (in other word can recognized wide range of carbohydrates [8].

II. Materials and Methods

A. Materials

Purified lectin (by ion exchange and gel filtration) Phaseolus vulgaris L. cv.white from local market , gluteraldehyde, bacterial suspension, spectrophotometer, brain-heart infusion broth and gram stain agents and pathogenic bacterial isolation as (Salmonella, Pseudomonas, Staphylococcus, Streptococcus, Morganella, Gardenella, Enterobacterium, Bacillus and Esheriesha . coli).

B. Methods

1) Bacterial suspension

Were grown on liquid media(Brain-Heart infusion broth) for 24h at 37C.

2) Agglutination

Assays were done on microtitere 50ml of lectin solution and the same amount of bacterial suspension.

3) Gluteraldehyde lectin immobilization [9]

Was made by mixing under mild agitation the polymerizing agent to the lectin solution (3part of lectin:1part of gluteraldehyde) .After 1 hour , the mixture was left at room temperature for 12 h, the lectins molicules formed a nonhomogenous insoluble polymer that was separated and wasked by succive centerfugations. 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.

4) Bacterial suspension-immobilized lectin interaction [10]

Was done by incubating at 37 ° C 1.5mL bacterial suspension with 0.5mL lectin suspension. OD at 660 nm was measured at the first moment of reaction (to) and after 10 minute of incubation (t10), while the tubes were not disturbed during incubation, to allow the polymer-cell aggregates to settle. This was an adaptation after touhami and col.

5) Slides

Stained by gram method were done from deposits of immobilized lectin-bacterial suspension and also from the immobilized lectin only (as control) and examined using an optical microscope.

III. Results

The results in the Table I showed strong agglutinations to the immobilized lectin with Bacillus, salmonella, staphylococcus, streptococcus and pseudomonas. With the immobilized lectin isolated from Phaseolus vulgaris L. cv.white more than the other Esheriesha. coli, Enterobacterium, Morganella and Gardenella that is very interesting in comparison with free lectin.

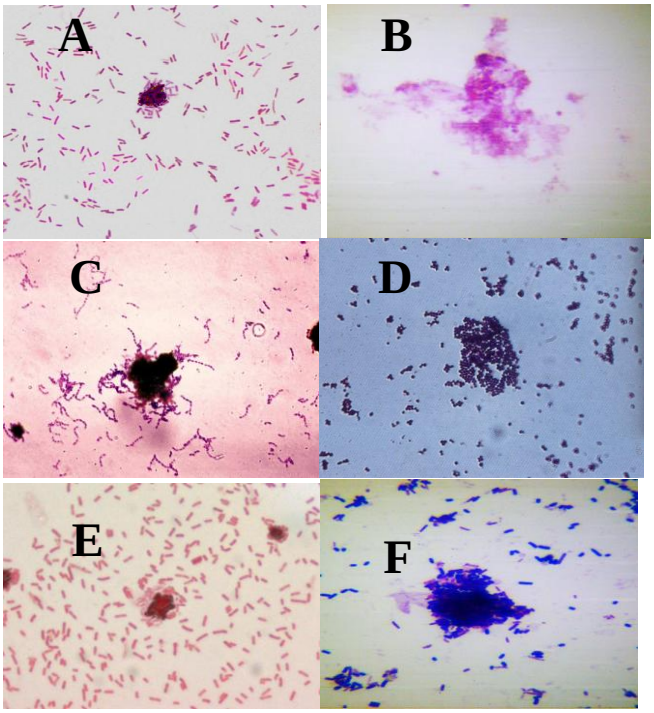

Fig. 1. B-Immobilized lectin(control), A-Pseudomonas sp. binding to immobilized lectin, C-Staphylococcus sp. binding to immobilized lectin ,D-Streptococcus sp. binding to immobilized lectin, E-Bacillus sp. binding to immobilized lectin, F-Salmonella sp. binding to immobilized lectin.

TABLE I: Variation of Od-660 Nm at the Start and after 10 Minute of Immobilized-Lectin Bacteria and Free-Lectin Bacteria Interaction

Bacterial isolation	T0	T10 (immobilized-lectin)	OD660 Δ (T10-T0)of immobilized-lectin	T10 (free-lecti)	OD660Δ (T10-T0)of free-lectin
E.coli	0.913	0.991	-	0.998	-
Bacillus	0.627	0.475	0.152	0.598	0.029
Enterobacterium	0.574	0.503	0.071	0.571	0.003
salmonella	0.617	0.452	0.601	0.601	0.016
Gardenella	0.744	0.758	-	0.759	-
staphylococcus	0.532	0.361	0.171	0.492	0.040
streptococcus	0.561	0.375	0.186	0.522	0.039
Morganella	0.408	0.531	-	0.549	-
pseudomonas	0.658	0.469	0.189	0.588	0.070

The agglutination pattern of Salmonella, Bacellus, staphylococcus and streptococcus with immobilized lectin

was presented in Fig. 1, it's important to notice that the tested lectin that efficiently agglutinated bacterial strain had a related carbohydrate specificity (N-acetylglucoseamine and its oligosaccharides) because this type of carbohydrate is the famous receptor on surfaces pathogenic bacteria[9].

This lectin was chosen because it agglutinated all the tested bacteria and its resistance to proteolytic attack in the gastrointestinal tract, was absorbed and induced systemic [6].

To improve the ability of immobilized lectin to agglutinate bacterial cell and settle it down and aggregate it togethor we stained the free lectin interaction with bacillus just to showed the ability of immobilized lectin to aggregating bacterial cell while the free lectin interacting with bacterial cell but no aggregation (Fig. 2) as shown with pseudomonas ssp. salmonella spp. with immobilized traditional lectin [3].

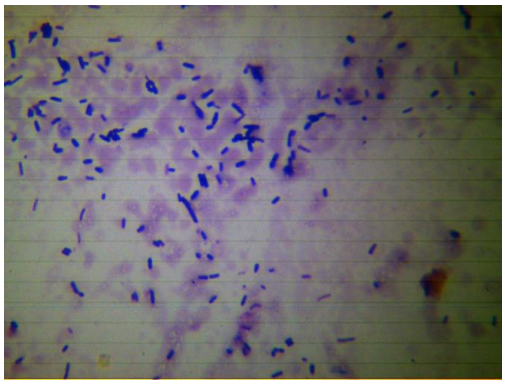

Fig. 2. Gram stained *Bacillus* binding with free lectin.

IV. Discussion

The interaction of the lectin polymer suspension with liquid culture of pathogenic bacterial cell was quantified by measuring at OD(660) nm at zero time and after 10 minute of standing, the decrease of the OD demonstrated that immobilized insoluble lectin polymer bound bacterial cells and the aggregates settled.

Lectins of phaseolus volgaris have a complex specifities of sugar so the reason of that results are:

1) The surface of the agglutinated bacteria contain carbohydrates receptors can recognized by white kidney bean
2) The surfaces of the non-agglutinated bacteria not contain the sugars receptors were recognized by white kidney bean.
3) The surface of the non-agglutinated bacteria may contain that specific carbohydrates receptors but not in the enough number.

As for the results of free lectin are: the lectin bind to cell surfaces but without precipitate them or inhibited them because the free lectin cannot Weighting the cell as the immobilized form also the free lectin cause no limitation to bacterial cell were attached to them as immobilized lectin as shown in Fig. 2 while Fig. 1 show the aggregation of bacteria with no motion.

They should be pointed that the interaction between bacterial cell and immobilized lectin is depending on the attachment of lectin with specific carbohydrate receptor on its cell surface.

These results suggest the possibility of plant lectins utilization for the preventation of some bacterial infection.

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