Blocking *Pseudomonas Aeruginosa*, *Chromobacterium Violaceum*, and *Ralstonia Solanacearum* Adhesion by Fruit Glycans

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Received: March 14, 2014; Accepted: April 23, 2014; Published: May 10, 2014

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Abstract

The soil-borne pathogens *Pseudomonas aeruginosa*, *Chromobacterium violaceum*, and *Ralstonia solanacearum*, possess the lectins PA-IL, PA-IIL, CV-IIL, RSL, and RS-IIL, which may mediate their adhesion onto animal and plant target cells, enabling infections. Such infections may be prevented by surrounding the sensitive cells with competing glycans, which act as glycodecoys that block patholectins and capture pathogens that bear them. The above-mentioned five lectins have been used by us as probes to reveal progeny-protecting glycodecoys in avian eggs, milk, royal jelly, and seeds. Herein we describe their usage as probes for fruit and onion glycodecoys. They revealed lectin-blocking galactosides, fructose, oligo/polysaccharides, and glycoproteins in most of the examined fruits. Galactose/arabinose-bearing compounds were detected by PA-IL in banana, carob, pineapple, pomegranate, kiwifruit, and dates. Diverse mannose/fucose-bearing compounds were detected by PA-IIL in banana, onion, and pomegranate; by CV-IIL in pineapple; by RSL in banana, carob, date, onion, and pineapple, and by RS-IIL in date and fig. The results show the high efficiency of these lectins as probes for natural infection-preventing glycodecoys. Usage of fruit and seed embryo-protecting glycodecoys, unless allergenic, is advantageous for preventing animal intestinal and external and plant wilting infections since they are natural, harmless, inexpensive, and widely available.

Keywords: Anti-infection; Fruit glycans; Soil-borne pathogens; *Pseudomonas aeruginosa*; *Ralstonia solanacearum*

Introduction

The worldwide *Pseudomonas aeruginosa* and tropical-subtropical *Chromobacterium violaceum* and *Ralstonia solanacearum* are soil saprophytic bacteria that aggressively decompose dead organisms and debris. Occasionally the first two are transformed into opportunistic animal (human) pathogens [1,2]. Clinical evidences describe these bacteria as a significant source of bacteria in burn victims, urinary-tract infections in catheterized patients, and hospital-acquired pneumonia in patients on respirators. *Ralstonia solanacearum* is well known devastating plant pathogen [3] wilting over 200 diverse species, causing heavy agricultural losses [4]. The emergence of these

bacteria as a major opportunistic pathogens might be the outcome of their resistance to the antibiotics and disinfectants.

P. aeruginosa produces two lectins: PA-IL (LecA) (that bind galactose [Gal] and L-arabinose [Ara]), and PA-IIL (LecB) (that binds fucose [Fuc] > mannose [Man] and fructose [Fru]) [5,6]. These lectins contribute to the pathogens adhesion to target cells [7] and amplify the destructive effects of their accompanying virulence factors on those cells [8]. They also contribute to the pathogen auto-aggregation into biofilms [6], increasing its adhesion to victim host tissues and hampering antibacterial agent penetration into its cells [9].

C. violaceum possesses a fucophilic lectin, CV-IIL, homologous to PA-IIL in structure and displaying similar specificity (Fuc > Man and Fru) with preferential selectivity for Fuc α 1,2- epitope [10].

R. solanacearum produces two lectins: a Man>Fuc-binding lectin, RS-IIL [11], homologous to PA-IIL in structure, and a fucophilic lectin, RSL [12,13], of special propeller-like structure [14]. These lectins may mediate the pathogen adherence to seedling cell-wall saccharides and xylem, leading to wilting infections.

Infections by these pathogens might be prevented by cell-surrounding glycans that act as decoys blocking the patholectins. Anti-adhesion therapies for bacterial infections offer an alternative mechanism of action which prevents the host harm [15,16]. Such glycodecoys are especially abundant around embryos and are supplied to neonates [17]. They include monosaccharides (e.g. seminal fluid and honey fructose protecting spermatozoa and bee larvae); disaccharides (e.g. milk lactose), and oligosaccharides bearing terminal Gal, Fuc, Man or Ara (in milk, saliva, seminal and other body/plant fluids). The oligosaccharides may be free or linked to short peptides, polysaccharides (PSs) or glycoproteins (GPs). The described knowledge might extrapolate to a wide range of clinical applications: inhibition of lectins - host receptors interaction, vaccination, use of food ingredients which involved in host-lectin interactions, composition of nature ingredient

and subminimal antibiotics concentrations and manipulation of hydrophobic glycodecoys [18-20].

Due to the crucial contribution of *P. aeruginosa* lectins PA-IL and PA-IIL to its pathogenicity, they have been widely used as authentic probes for searching natural [6] and synthetic [21-23] glycodecoys for blocking the pathogen binding to target cells. PA-IL and PA-IIL were highly useful for glycodecoy detection in human body fluids [6], avian egg whites [24,25], milks [26,27], royal jelly and honey [28]. Further on, CV-IIL, RSL, and RS-IIL were added to the scanning lectin battery -for glycodecoy search in those animal substances, floral nectars, and edible seeds [6,29-31].

The current study aim was glycodecoy detection in edible fruit and onion extracts for the following purposes: 1) exhibition of the universality of "glycodecoy contribution to endogenous embryo protection against infections"; 2) application of the authentic patholectins as probes for discovering natural glycodecoys; 3) determination of natural glycodecoy protective capacity; and 4) choosing the best plant formulas for seedling protection against *R. solanacearum* and animal protection against *P. aeruginosa* gastrointestinal [32] and external infections. PA-IL, PA-IIL, CV-IIL, RSL, and RS-IIL - blocking by non-dialyzed and dialyzed fruit extracts, differentiating between low (<10 kDa, LMM) and high molecular mass(>10 kDa, HMM) glycans was examined using hemagglutination-inhibition (HAI) test. The extracted GPs were analyzed by Western blotting (Wb) using the five bacterial lectins labeled by horseradish peroxidase.

Results

All the fruit and onion extracts inhibited (diversely) the five patholectin hemagglutinating activities (Figures 1 and 2, top panels). Inhibiting activities due to LMM (<10 kDa) components were removed by dialysis, retaining the HMM activities.

As seen in Figure 1, PA-IL was blocked by most of the fruit LMM and HMM (PSs and GPs) components (top panels). It was most strongly blocked by banana, carob, pineapple, and pomegranate, followed by kiwifruit, while least sensitive to blocking by onion extract. It also stained their GPs (Wbs in the respective bottom panels), most strongly and widely the date GP bands (around 10 heavy bands at 25-105 kDa), followed by pineapple (1 heavy band at around 25 kDa and a compact band-cluster at 150-250 kDa), carob and onion (about five GP bands each at 40-105 kDa), fig (a sole band at 25 kDa), kiwi (2 bands at 20-30 kDa), and terminating in pomegranate (1 pale GP band). It did not stain any banana or grape GP.

Figure 1 shows that PA-IIL, was more sensitive than PA-IL to all the extracts examined. It was most strongly blocked by pomegranate, grape, and kiwifruit, followed by date, carob, banana, and pineapple. It also nicely reacted with onion and fig. Like PA-IL, PA-IIL strongly stained a wide spectrum of the fruit extract GPs, excluding grapes. Unlike PA-IL, it did react with banana and pomegranate GPs, displaying considerably more pronounced staining of onion and pomegranate GP bands.

CV-IIL sensitivity to the fruits was considerably weaker, and

unlike the other lectins, it was not inhibited at all by onion (Figure 1, top panel). It was most sensitive to inhibition by kiwifruit and pineapple, followed by pomegranate, grape, carob, and date. CV-IIL stained only few fruit GPs (Figure 1, bottom panel), most strongly those of pineapple.

As seen in Figure 2, the *R. solanacearum* lectin RSL, was quite sensitive to blocking by the fruit glycans (top panel) being most strongly blocked by extracts of pomegranate, followed by date, kiwifruit, grape, pineapple, and carob. This lectin strongly stained many GPs (ranging from 15 to 250 kDa) in the date, onion, carob, banana, and pineapple Wbs (much more than the other lectins), but no grape and negligible fig GP bands were stained by it.

Figure 2 further shows that RS-IIL was the most sensitive lectin to the fruit extracts, being most strongly inhibited by all of them and unique for its strongest interactions with the fig

Figure 1: Interactions of fruit and onion extracts with *P. aeruginosa* galactophilic and fucophilic lectins PA-IL and PA-IIL (respectively) and *C. violaceum* fucophilic lectin CV-IIL. The interactions were examined by inhibition of the lectin hemagglutinating activities by nondialyzed and dialyzed samples of the extracts (top panels) and by Western blotting (Wb) using the same lectins labeled by peroxidase (bottom panels).

Figure 2: Interactions of fruit and onion extracts with *R. solanacearum* mannophilic (>fucophilic) RS-IIL and fucophilic (>mannophilic) RSL lectins. The interactions were examined by inhibition of the lectin hemagglutinating activities by nondialyzed and dialyzed samples of the extracts (top panels) and by Western blotting (Wb) using the same lectins labeled by peroxidase (bottom panels).

extract glycans (which reacted most weakly with the other lectins), including special staining of the fig 25-80 kDa GPs. RS-IIL was also most strongly blocked by kiwifruit, pomegranate, and grape, followed by banana, pineapple, and date, and it also showed a clear sensitivity to carob and onion. This lectin stained many date, carob, and banana GP bands, in addition to the unique rich staining of the fig GPs. Like the other lectins, it also did not stain grape GPs, while staining few kiwi GPs. Unlike PA-IIL and RSL, RS-IIL stained only a few onion GPs and was profoundly outstanding in not staining the pineapple HMM GP cluster (75-250 kDa), which was clearly stained by the other four lectins.

Discussion

Prevention of pathogenic bacterial infections in animals and plants is a major goal in medicine, veterinary medicine, and agriculture. Since most pathogenic bacteria have become antibiotic-resistant, special efforts are invested in preventing infections by hampering their adhesion to the target cells.

Natural anti-infective tactics hamper lectin-dependent pathogen binding to cells by surrounding the sensitive cells with glycodecoys that compete with the cell receptor saccharides [32-34]. These glycodecoys not only block lectin binding to the sensitive target cells, but also capture the lectin-bearing bacteria, preventing their adhesion to the target cells via additional adhesins. This strategy protects immuno-immature animal and plant embryos, neonates, and sensitive adult cells (including

germ cells) against infections.

The patholectins PA-IIL and PA-IIL of *P. aeruginosa*, CV-IIL of *C. violaceum*, and RSL and RS-IIL of *R. solanacearum*, were shown by us during the last decade to be highly useful authentic probes for detection of glycodecoys in avian egg whites [6,24,25], milk [27] royal jelly and honey [6,28] and seed glycans [29-31] (Table 1).

The present report describes their usage for the study of plant-glycodecoy presence in extracts of eight fruits and onion. The fruits were chosen because they harbor seeds, they contain non-starch PSs that bear glycodecoy-type glycans (their LMM and multivalent polymeric glycans, including PSs and GPs, fit that function), they are not expensive, and are widely consumed food stuffs, generally without adverse effects, except allergies [35-37].

Each fruit extract was examined without fractionation for determination of total lectin- blocking activities of both LMM and HMM glycans, and following removal of LMM (<10 kDa) by dialysis – for evaluation of the contribution of the retained HMM fraction to lectin blocking.

While leguminous seed glycans generally inhibited PA-IIL more strongly than the Fuc/Man-binding patholectins [29] the fruit and vegetable extracts inhibited the other four lectins more than PA-IIL.

The concentration of potential PA-IIL-blocking monomeric Gal and Ara in fruits is very low: grapes contain 7.5-16, kiwi 27, and onion 15 mg Gal/100 g fresh weight. However, the Gal-containing disaccharide melibiose (Gal α 1, 6Glc), trisaccharide raffinose (Gal α 1,6Glc β 1,2Fru), and tetrasaccharide stachyose (Gal α 1,6 Gal α 1,6Glc β 1,2Fru) are abundant. Raffinose is one of the most abundant trisaccharides in nature, widely occurring in plants (like sucrose), and stachyose coexists with them [38]. These saccharides are strong inhibitors of PA-IIL: melibiose, raffinose, and stachyose are x13.3, x4.4 and x5.7, respectively, more potent inhibitors of PA-IIL than monomeric Gal [39]. The Gal/Ara-bearing PSs: galactomannans, or galactoglucomannans (Table 2 [2]) and arabinogalactans, which are potent PA-IIL inhibitors, are major fruit primary cell-wall PSs existing in plants in high “concentrations”: “xyloglucans”, which are comparatively homogeneous PSs made of glucan backbone substituted with xylosyl side chains, also occasionally bear galactose residues (Table 2 [5]). GPs that coexist with them at low concentrations only weakly contribute to PA-IIL blocking in plants, contrary to their major function in animal embryo- and neonate- protection [6,24]. The Wb data (Figure 1, bottom panels) show that PA-IIL-inhibiting activities of banana, grape, and pomegranate HMM glycans are not dependent on their GPs since no band-staining with PA-IIL was observed in their Wbs. The GPs of the other fruits attracted PA-IIL, which moderately stained a few carob and onion GP bands, while very strongly and widely staining around 10 heavy date GP bands, but only few fig and kiwifruit bands.

PA-IIL and CV-IIL inhibitors include free fructose, mannosylated and fucosylated PSs, and GPs possessing Man and Le (a) or H epitope-bearing antennae (Table 2). The last two

Table 1: Inhibition of the hemagglutinating activities of the five patholectins by avian egg white, milk, royal jelly, and seed glycodecoys – all of them protecting sensitive animal offsprings (presented as HAI log₂ dilution⁻¹).

Embryo/neonate- protecting medium: Lectin	PA-IL	PA-IIL	CV-IIL	RSL	RS-IIL
Chicken egg white	0	4	3	1	4
Quail egg white	0	15	23	6	22
Pigeon egg white	22	16	15	4	19
Human milk	8	19	8	15	10
Cow milk	7	1	2	3	4
Royal jelly	6	14	11	6	13
Seeds* (cashew, cocoa, coffee, pumpkin)	7.5	6	1.2	4	11.5

*The values are means of the four seeds. Highest HAI values are in bold.

epitopes are strong ligands of PA-IIL and CV-IIL, respectively.

Fructose, which blocks PA-IIL, CV-IIL, RSL, and RS-IIL, exists in fruits as a monomer and as the most abundant disaccharide sucrose. Mannose is rare as a monomer; there are limited amounts of fruit mannans, and oligomannosylated N-glycans are present only in minor amounts [40,41]. The contribution of the plant GPs to this lectin-blocking is low as opposed to the mannosylated animal GPs (e.g., quail egg albumen and royal jelly) [25,28], which strongly block PA-IIL.

Fuc, which also blocks the same four lectins, is present in plant PSs as Fuc α 1,2- [42] linked to Gals of xyloglucans, which occasionally bear galactose or fucosyl-galactose residues (Table 2 [5]). It exists in GPs as core Fuc α 1,3- bound to Man(2) Xyl-bearing N-glycans (MUX, Table 2 [7,8]), which strongly attract RSL, or as a single Fuc α 1,4- [Le](a) epitope that strongly attracts PA-IIL (Table 2 [13]). PA-IIL and RSL may also bind to complex N-glycans bearing both Le(a) and core Fuc α 1,3/6- branches (Table 2 [12]). Among the mannosylated/fucosylated GP N-glycans, the most abundant ones bear a β 1,2-xylosyl and a core alpha 1,3-Fuc residue linked to the terminal N-acetylglucosamine, similar to those of the major N-linked oligosaccharides of horseradish peroxidase and pineapple stem bromelain (Table 2 [7,8], MUXF and MMXF).

The sensitivity of *R. solanacearum* lectin RS-IIL to the fruit glycans was highest (Figure 1,2), like the outstanding sensitivity of PA-IIL to human milk [43,44]. Including the mannophilic plant lectin Con A with the bacterial lectins (Table 3) shows that PA-IIL and RS-IIL share with it insensitivity to the plant galactomannans vs. high sensitivity to yeast mannan. RS-IIL is closer to Con A than to PA-IIL in its lower sensitivity to human milk GPs and its insensitivity to the milk Le (a) epitope.

Taking the kiwifruit (*Actinidia deliciosa*) as an example, Schroder et al. [45] reported that the galactoglucomannan from the primary cell walls of the ripe fruit is mainly composed of alternating Glc β 1,4 Man backbone, with approximately one third of it carrying either single Gal α 1,6 or Gal β 1,2 Gal α 1-. Its neutral arabinogalactan (7%) was proposed to contain a highly branched galactan with alternating 1,3- and 1,6-Gal backbone and 1,3- and 1,6-Gal side chains terminating with 1,2-Ara residues. Its major

(93%) acidic arabino rhamnogalacturonan was shown to be mainly composed of rhamnogalacturonan backbone-bearing terminal 1- α -L Ara and Gal residues as side chains [46,47]. These fruit GPs also bear oligosaccharide antennae with terminal Gal, Man, and Fuc (Table 2), including the Le (a) epitope [35,48,49], who diligently examined plant-glycan involvement in food allergies and cross-allergic reactions between diverse plant products, described data of 27 plant foodstuffs, showing that the glycans of these foodstuffs ranged from the typical vacuolar "horseradish peroxidase" type and oligomannosylated types (Table 2 [8 and 9]) to complex Le(a)-carrying structures (Table 2 [11,12]). Apple, banana, carrot, kiwi, and onion, as well as other fruits (out of the scope of our research), were found by them to be particularly rich in Le (a)-carrying N-glycans. They concluded that the Le (a)-type structure is very widespread among plants, excluding cauliflower. The presence of Le (a) in fruits resembles its presence in human milk. Its outstandingly high affinity to PA-IIL [43,44] is in agreement with the view that it might contribute to the protection of the fruit embryos against PA-IIL-bearing *P. aeruginosa*, as does human milk. Comparison of the fruit and onion patholectin-inhibiting efficiencies towards the five lectins (Figures 1 and 2, top panels and Table 3) has shown that pomegranate and kiwi, followed by pineapple, grapes, and dates, possess the overall strongest patholectin-blocking activities. Banana and carob, followed by pineapple, pomegranate, and kiwifruit, were most efficient for blocking PA-IL; pomegranate and grapes, followed by kiwi and dates, were most efficient for binding PA-IIL; kiwifruit and pineapple, followed by pomegranate, were most effective CV-IIL inhibitors; and pomegranate, followed by dates, kiwi, and grapes, were the strongest RSL inhibitors. RS-IIL was unique in being most sensitive to fig glycans, followed by those of kiwi, pomegranate, grapes, banana, and pineapple. Comparison of the fruit interactions with the diverse lectins showed a very interesting analogy between banana and carob extracts in regard to the two *P. aeruginosa* lectins and Con A (Table 3). Both most strongly blocked PA-IL. Their identical inhibitions of PA-IIL (Table 3) were probably due to their fructose, Man-bearing compounds, and Fuc-bearing Le (a) [35]. Wilson et al. documented the presence of Le (a) in banana. The banana and carob interactions with the other four lectins diverged: banana exerted a stronger inhibition of the mannophilic

Table 2. Proposed structures of natural PSs and N-glycans that block the five patholectins.

Name (abbreviation)	Proposed Structure
1) Galactosylated pectin (GalA = galacturonic acid) (Rha = rhamnose)	–D-GalA α 1,2 L-Rha α 1,4D-GalA– $\uparrow$ 1,4 Galβ $\pm$ L-Ara α 1,3/6
2) Galacto/galactogluco-mannan	–Man β 1,4Man β 1,4(Man β 1,4) _n Man– /Man β 1,4Glc β 1,4Man– $\uparrow$ 1,6 Galα Galα $\pm$ Gal /Xyl/Ara1,3/6
3) Arabinogalactan (GlcA = Glucuronic acid)	–Gal β 1,3Gal β 1,3(Gal β 1,3) ₂ Gal β 1–3Gal– $\uparrow$ 1,6 $\uparrow$ 1,6 Ara α 1,3Gal α Gal α $\uparrow$ 1,6 $\uparrow$ 1,6 Ara α 1,3Gal α Ara α $\uparrow$ 1,6 $\uparrow$ 1,5 Galα/GlcA Araα
4) Mannan	–6Man α 1,6Man α 1– $\uparrow$ 1,2 $\uparrow$ 1,2 Manα Man α $\uparrow$ 1,2 Manα1,3Manα
5) Fucosylated xyloglucans	–Glc β 1,4Glc β 1,4 Glc β 1,4 Glc β 1,4–;–Glc β 1,4Glc β 1,4 Glc β 1,4 Glc β 1,4– $\uparrow$ 1,6 $\uparrow$ 1,6 $\uparrow$ 1,6 $\uparrow$ 1,6 $\uparrow$ 1,6 $\uparrow$ 1,6 Xyl α Xyl α Xyl α Xyl α Xyl α Xyl α $\uparrow$ 1,4 $\uparrow$ 1,2 $\uparrow$ 1,2 Gal α Gal β Gal β $\uparrow$ 1,2 $\uparrow$ 1,2 Fuca Fuca
6) Oligo (3) mannosylated N-glycan	Manα1,6 \ Man β 1,4GlcNAc β 1,4 GlcNAc β 1–Asn Manα1,3 /
7) Bromelain–type N-glycan MUXF ³	Manα1,6 \ Man β 1,4GlcNAc β 1,4GlcNAc–Asn Xyl β 1,2 / $\uparrow$ 1,3 Fuca
8) Horseradish peroxidase–type N-glycan MMXF ³	Manα1,6 \ Manα1,3 – Man β 1,4GlcNAc β 1,4GlcNAc–Asn Xyl β 1,2 / $\uparrow$ 1,3 Fuca
9) Highly (9) mannosylated N-glycans	Manα1,2 Man α 1,6\ Man α 1,6\ Manα1,2 Man α 1,3/ Man β 1,4GlcNAc β 1,4 GlcNAc β 1–Asn Manα1,2 Man α 1,2Man α 1,3/
10) H blood group trisaccharide	Fuca1,2 Gal β 1,3/4GlcNAc –
11) Lewis(a) tetrasaccharide	–Gal β 1,3GlcNAc1,3Gal – $\uparrow$ 1,4 Fuca
12) Lewis(a) and core Fuca1,3/6	Galβ $\downarrow$ 1,3 Fuca Fuca1,4 GlcNAc β 1,2Man α 1,6 \ $\downarrow$ 1,6 Man β 1,4GlcNAc β 1,4GlcNAc–Asn Fuca1,4 GlcNAc β 1,2Man α 1,3 / $\uparrow$ 1,2 $\uparrow$ 1,3 Galβ Xyl β Fuca

RS-IIL while carob was a stronger inhibitor of the fucophilic CV-IIL and RSL. The banana and carob Wb data (presented in Figures 1,2, bottom panels) seem to indicate that the blocking terminal Gal α 1-6 residues in banana are merely associated with oligosaccharides and PSs rather than with GPs, since no PA-IL-stained GP bands were observed in the banana extract Wb. This was in contrast to the clear PA-IL staining of the carob and several other fruit GPs, and as opposed to the staining of banana GP bands by the other four lectins (Figures 1,2, bottom panels). Hence, it may be deduced that the contribution of banana, as well as grape and pomegranate GPs, to PA-IL blocking, is negligible (Figure 1, bottom panel). However, in contrast to the total absence of GP bands in grapes, banana GPs are stained by the other four lectins. Two banana GP bands (at around 28 and 34 kDa), which were selectively stained by CV-IIL, and also strongly by PA-IIL and RSL, might bear H-type Fuc α 1,2 epitope (which is an important ligand of these three Fuc- specific lectins). A third GP band (at around 50-55 kDa) strongly stained by PA-IIL and RSL, can bear the Le (a) epitope, which had already been documented [35,36] and core Fuc, respectively. The banana Wbs showed additional GPs (at a range of around 40 to 50 kDa) that were strongly and selectively stained by RSL (probably due to core Fuc3) and additional GPs that were more weakly stained by PA-IIL, RSL, and RS-IIL. Despite stronger blocking of RSL by carob than by banana, their Wbs with RSL seemed to be equal (at a range of 30 to 250 kDa), differing in the molecular mass distribution of the more conspicuous GP bands. The extracted date and fig glycans shared relatively high affinity to the mannophilic RS-IIL and low PA-IL binding, but differed in their interactions with the other three fucophilic lectins and in their Wbs. As may be seen in Figures 1 and 2 (top panels) and Table 3, the fig-extract blocking of PA-IIL, CV-IIL, and ConA were weaker than those of the date. The Wbs of these 2 fruit extracts were also very interesting (Figures 1,2, bottom panels). The date GP glycans, as compared to the other eight fruits, displayed strongest, richest and widest GP band reactivity with all five patholectins – indicating the presence of both terminal Gal (PA-IL-stained) and Fuc/Man (stained by the four lectins) residues. The fig extract blocking of the five lectins is probably due to their fructose, galactosylated and mannosylated oligosaccharides, and PSs, which selectively most strongly blocked the mannophilic RS-IIL.

However, in contrast to dates, its Wbs were relatively poor: only the RS-IIL-stained Wb showed considerable GP band staining, while only one GP band was strongly stained by PA-IL and CV-IIL. The high sensitivity of RS-IIL to fig N-glycans seems to indicate the presence of an important RS-IIL ligand on them. This is a significant preliminary finding deserving further study. The grape-extract strongest blocking of PA-IIL, RS-IIL, and Con A, followed by RSL, is very similar to the date pattern (Table 3). This may be due to fructose and mannosylated PSs but not GPs since, in contrast to the rich date GP staining, no GP bands were observed in any of the grape Wbs (Figures 1 & 2 bottom panels). The grape-negative Wbs, with all the lectins, were outstanding among the herein-examined fruits but similar to our previous results with cocoa-seed extracts [30,31]. Kiwifruit and pineapple represent another pair of fruits being similar in their very strong interactions with almost all the examined lectins and with special preference for CV-IIL (Figures 1 & 2 Table 3). The pineapple inhibition of PA-IL is stronger, and of PA-IIL –weaker than those of the kiwifruit, which was reported to contain Le (a) epitope [35]. Strongest inhibition of CV-IIL (Table 3) could be attributed to fructose, oligosaccharides, and H-type Fuc α 1,2-bearing xyloglucans since their Wbs presented relatively poor lectin-stained GP band repertoire. Two GP bands in the kiwi and one band in the pineapple (at a range of 20 to 30 kDa) were stained by the five patholectins (Figs. 1,2 bottom panels). In addition, in the pineapple, there was a cluster of bands at 75-250 kDa equally stained by CV-IIL, PA-IL, and PA-IIL, with stronger staining by RSL but almost no staining with RS-IIL. The strong PA-IL blocking activity of kiwi and pineapple might be mainly attributed to their above-described Gal/Ara-containing PSs [46,47]. Figures 1,2 show another very interesting observation – strongest overall lectin- blocking activities of the pomegranate without considerable GP contribution – versus the weakest overall lectin-blocking of the onion (not inhibiting CV-IIL and only weakly inhibiting PA-IL and RSL) apart from strong blocking of PA-IIL, followed by RS-IIL, associated with rich staining of its GP bands by both PA-IIL and RSL (at a range of 20 to 105 kDa). The combined PA-IIL and RSL binding to the onion GPs is in accord with the description of N-glycans that bear both core Fuc and Le (a) (Table 2[12]), and in accord with the Wilson et al. report on Le (a) epitope presence in onion N-glycans. Since its GP bands were

Table 3: Comparison of the relative inhibitory potential of each of the eight fruit and onion extracts for the five patholectins (with ConA as a reference mannophilic lectin).

	PA-IL	PA-IIL	CV-IIL	RSL	RS-IIL	Sum	ConA
Banana	100	80	30	35	75	320	55
Carob	100	80	55	05	42	327	55
Date	55	90	50	80	58	333	98
Fig	55	60	40	35	100	290	45
Grapes	60	100	52	54	90	358	76
Kiwi	78	96	100	56	95	425	47
Onion	32	70	0	23	42	167	43
Pineapple	95	85	100	53	60	393	45
Pomegranate	91	100	70	100	95	456	100

also weakly (identically) stained by PA-IL and RS-IIL, it seems that they also possess Gal- and Man/Fuc-bearing N-glycans. Taken together, the data of the herein presented research of fruit and onion glycodecoys by means of five authentic patholectins (two of *P. aeruginosa*, one of *C. violaceum*, and two of *R. solanacearum*) add important information related to plant embryo-protection against infections, which is also relevant to humans, other animals, and plants. Owing to the selective glycan specificities of the five patholectins, they have been proven to be powerful probes for the identification of diverse anti-infective glycodecoys in diverse organisms. Comparing the lectin binding by the plant and animal glycodecoys has enriched the perspective of this subject. Blocking PA-IL, PA-IIL, RSL, and RS-IIL by common epitopes of human milk, avian egg whites, beehive products, and fruits/onion most strongly indicates the importance of their consumption as food and their medical, veterinary and agricultural usage. Although almost all the examined fruit extracts inhibited the five lectins, with pomegranate displaying the highest patholectin-blocking activity, followed by kiwifruit, pineapple, grapes, and fig, PA-IL was most strongly blocked by banana and carob and RS-IIL was outstanding in its highest affinity to the fig, followed by kiwi, pomegranate, and grapes. The present results seem to indicate that in fruits as in the animal-protecting systems, both LMM and HMM glycans are important for blocking patholectins. However, in animals, the GP contribution is higher while in plants, the PSs are important. In addition to their efficiency as glycodecoy probes, the five patholectins might also be useful probes for detecting allergenic glycans. The plant embryo-protecting glycodecoys, unless allergenic, are advantageous for preventing animal (intestinal and external) and plant infections since they are natural, widely available, and consumed as mostly harmless, inexpensive edible plant products.

Experimental Procedures

Lectin preparations

The bacterial lectins: PA-IL, PA-IIL, CV-IIL, RSL, and RS-IIL, were isolated from cell extracts of *P. aeruginosa* ATCC 33347, *C. violaceum* (Bergonzini) ATCC 12472, and *R. solanacearum* ATCC 11696, purchased from the American Type Culture Collection (ATCC) (Manassas, Virginia), and purified, as described earlier [5,10-12]. Quality controls of the purified lectins were performed by SDS-PAGE with Coomassie brilliant blue staining.

The fruit and onion extract preparations

Extracts of fresh banana (*Musa acuminata*), carob (*Ceratonia siliqua*), date (*Phoenix dactylifera*), fig (*Ficus carica*), grape (*Citrus paradisi*), kiwi (*Actinidia chinensis*), pineapple (*Ananas comosus*), pomegranate (*Punica granatum*) and onion (*Allium cepa*) were prepared by their chopping, suspension at 10% (V/V) concentration in 0.025 M phosphate buffered (at pH 7.2) isotonic saline (0.85% NaCl) solution (PBS), and homogenization in a food processor. Each suspension was centrifuged (10,000g) for 10 min and the supernatant fluid was carefully collected. A half-volume of each supernatant was dialyzed overnight (using a dialysis membrane cut-off of 10 kDa) against x500 volume of PBS at pH

7.2 (refreshed three times) at 5°C. Both the non-dialyzed and dialyzed preparations were divided into several 1-mL aliquots for storage at 5°C for a few days or at -20°C for longer periods.

Hemagglutinating activity (HA) determination: The lectin HA was determined using papain-treated human type O (H) red blood cells (erythrocytes). The erythrocytes (kindly obtained from the Magen David Adom National Blood Services in Israel) were washed 3 times with PBS, and then treated with 0.1% papain with 0.01% cysteine, as previously described [5]. A 50-μL sample of each bacterial lectin preparation examined (starting in 20 μgmL⁻¹ solution) was serially 2-fold diluted in tubes with 50 μL of saline. Then, saline and 5% (V/V) suspension of the papain-treated erythrocytes in saline (50 μL each) were added to each tube and thoroughly mixed. After 30 min at room temperature, the tubes were centrifuged for 30 sec (1000 ×g), and HA was examined, as previously described [5]. The highest dilution leading to agglutination of all the erythrocytes in one mass was determined. The activity was represented by the number of tubes (2-fold dilutions) in which there was hemagglutination ($\text{Log}_2 \text{dilution}^{-1} = 7$ means 7 positive tubes = positive reaction up to dilution of 1:128, or original activity of 128 hemagglutination (units).

Hemagglutination inhibition (HAI) test: In the HAI test, each seed extract was serially 2-fold diluted in 50 mL of saline. Then 50 mL of the lectin solution (at highest dilution that agglutinates all the erythrocytes in one large mass) was added to each tube. After 30 min at room temperature, 50 mL of the papain-treated human O type erythrocyte suspension was added to each tube and HA was examined, as described above [5]. HAI intensity was represented by the number of 2-fold dilutions ($\text{Log}_2 \text{dilution}^{-1}$) without considerable hemagglutination preceding its reappearance.

Western blot (Wb) analyses: Wb analyses demonstrating the differential peroxidase-labeled lectin interactions with individual SDS-PAGE-separated GPs, were performed as follows: 15-μL samples of each fruit/onion extract at a concentration of around 1 mg.mL⁻¹ were mixed 1:1 with a sample buffer, boiled, and applied to wells in 10% SDS-PAGE (at 140 V) in a Mini-PROTEAN Cell 3 Electrophoresis (Bio-Rad, Hercules, California), as previously described [43]. Following SDS-PAGE, the proteins were transferred to a nitrocellulose (0.45 μm, Bio-Rad) membrane at 4°C for 2 h (85 mA/40-50 V) using the Mini Trans-Blot Module (Bio-Rad). The membranes were incubated overnight in blocking buffer 0.02 M, PBS pH 7.2, containing 3% bovine serum albumin and 0.05% Tween 20, exposed to horseradish-peroxidase-labeled lectins (about 1 μg/mL⁻¹, dissolved in the same blocking buffer with 0.1% Tween 20) at room temperature for 2 h, and then thoroughly washed. The peroxidase reaction was visualized using enhanced chemiluminescence (Amersham International PLC, Buckinghamshire, UK) and recorded onto photographic films. Controls with the peroxidase-labeled lectins in the presence of a 0.3 M concentration of the relevant blocking sugars in their reaction mixtures were used in parallel in order to rule out nonspecific (sugar-independent) lectin binding [43].

Statistical analysis: The HAI results were analyzed by Student's t-test. The data presented in the figures generally represent means $\pm$ SE (standard errors of the means) of at least five experimental results for each lectin.

Acknowledgements

The authors express their gratitude to Ms Sharon Victor for her great help with editing and preparation of this manuscript and to Ms Ela Gindy for her great help in the graphic presentation. This work is part of the PhD thesis of Ofra Rachmaninov, Bar-Ilan University.

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