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Interaction Between Plant Agglutinins and *Legionella* Species

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Serogroups of *Legionella pneumophila* exhibited differential reactivities with plant agglutinins. Agglutination patterns were modified by growing the organisms in different media. The passage of four strains through guinea pigs did not result in altered reactivities with lectins or with plant agglutinins.

The *Legionellaceae* organisms are aerobic, flagellated, gram-negative rods which have an abundance of branched chain fatty acids. The organisms exhibit long wavelength UV fluorescence and produce pigments, catalase, and gelatinase, but do not grow on blood agar. Additional characteristics such as DNA homology, hippurate hydrolysis, and oxidase and β-lactamase production are frequently used in the further classification of the bacteria (1, 5, 7, 10, 11, 15). *Legionella pneumophila* can be classified by serogroup (8). There are at least six distinct serological groups of *L. pneumophila*, but the antigenic compositions of the group antigens remain unknown. We have previously shown that lectins and plant agglutinins can be of great value in the laboratory identification of *Neisseria* (12) and *Staphylococcus* (2) species. Lectin probes are also useful in studying the structure of individual cell surface polymers such as teichoic acids (3). In this note, we show that plant agglutinins gave differential reactivities with many of the serogroups of *L. pneumophila* and some of the other *Legionella* species. The results may serve to further characterize the legionellae.

Stock *Legionella* strains used in this study were obtained courtesy of R. Weaver (Centers for Disease Control, Atlanta, Ga.). The animal-passed strains of Knoxville, Togus, Los Angeles, and Chicago were obtained in our laboratory by passage through weanling guinea pigs (one to five consecutive passages) after intraperitoneal injection of 1 ml of bacterial suspension. Bacteria were reisolated from heart blood or peritoneal exudate fluid or both plated on buffered charcoal-yeast extract (CYE) agar (13). The passaged bacteria were virulent in guinea pigs; lethality was used as a criterion for virulence at 10^8 cells per ml. Before animal passage, cell densities of $>10^{12}$/ml were required to kill guinea pigs. Stock cultures were maintained on GC-FC agar (GC medium base [Difco Laboratories] plus L-cysteine and ferric pyrophosphate [14]) or CYE agar at 37°C in an atmosphere of 5% CO_2 and transferred weekly. The animal-passed strains were maintained as frozen stocks and transferred once on CYE agar before use.

For experimental purposes, bacteria were grown for 40 ± 2 h at 37°C in 5% CO_2 on CYE agar or on GC-FC agar plates. The cells were washed from the plates with phosphate-buffered saline (PBS; 50 mM sodium phosphate–150 mM sodium chloride, pH 7.3). The suspensions were washed twice in PBS and finally adjusted to an optical density of 1.0 at 450 nm (1-cm path length). Samples (50 μl) of the buffered bacterial suspensions were then mixed with 50 μl of lectin or plant agglutinin (1.0 μg/μl) in round-bottom microtiter wells. After an overnight incubation at 4°C, the wells were examined for evidence of agglutination reactions. Lectins were purchased from E-Y Laboratories, San Mateo, Calif. The agglutinins from *Persea americana* and *Mangifera indica* were isolated by the method of Meade et al. (9). The *Persea* and *Mangifera* preparations do not contain significant amounts of protein and are referred to as plant agglutinins rather than as lectins (6, 9). The agglutinin from *Aloe arborescens* was isolated by the method of Fujita et al. (4). The agglutinating activity of *Albizzia julibrissin* was obtained by extracting the finely ground seeds with PBS and then retaining the fraction which was soluble in 40% saturated ammonium sulfate, but insoluble in 70% saturated salt. All agglutinins were stored in a freeze-dried form and dialyzed against PBS before use.

The results are summarized in Table 1. The patterns of agglutination vary between all of the serogroups of *L. pneumophila*. The agglutination patterns do not, however, reflect serogroup-specific trends, since it was observed that all five strains of serogroup 1 were unique with respect to agglutination by the four agglutinins. When several well-characterized lectins (6) such

TABLE 1. Interactions between plant agglutinins and *Legionellaceae*[a]

Bacterium	Agglutinin	*Aloe arborescens*		*Mangifera indica*		*Persea americana*		*Albizzia julibrissin*	
		CYE	GC-FC	CYE	GC-FC	CYE	GC-FC	CYE	GC-YC
L. pneumophila	Bellingham-1 [1]	+	+	+	−	+	+	−	−
L. pneumophila	California-1 [1]	−	−	−	−	−	+	−	−
L. pneumophila	Knoxville-1 [1]	+	−	−	+	+	+	−	−
L. pneumophila	Philadelphia-2 [1]	+	+	+	+	+	+	−	−
L. pneumophila	Pontiac-1 [1]	+	+	−	+	+	+	−	−
L. pneumophila	Togus-1 [2]	+	−	+	+	+	+	+	−
L. pneumophila	Bloomington-2 [3]	+	−	+	+	+	+	−	−
L. pneumophila	Los Angeles-1 [4]	−	−	−	−	−	−	−	−
L. pneumophila	Dallas-1E [5]	+	+	+	+	+	+	+	−
L. pneumophila	Chicago-2 [6]	+	+	+	+	+	+	+	+
L. bozemanii		−	−	−	−	−	−	−	−
L. dumoffii		+	+	−	−	−	−	−	−
L. gormanii		−	ND	−	ND	−	ND	−	ND
L. micdadei		−	ND	−	ND	−	ND	−	ND

[a] Numbers in brackets refer to serogroup; +, agglutination; −, no agglutination; ND, not determined owing to failure of the species to grow in the medium.

as *Arachis hypogaea, Bandeiraea simplicifolia* (I and II), *Bauhinia purpurea*, concanavalin A, *Dolichos biflorus, Glycine max, Helix aspersa, Helix pomatia, Maclura pomifera, Phaseolus limensis, Phaseolus vulgaris, Pisum sativum, Ricinus communis* (I and II), *Robinia pseudoacacia, Salvia horminum, Salvia sclarea, Solanum tuberosum, Sophora japonica, Ulex europaeus*, and wheat germ agglutination were employed in the assays, no evidence for agglutination was observed for any of the bacteria. The agglutination patterns were dependent on the origin of the cells, because the agglutinin reactivities of bacteria obtained from CYE agar plates were frequently different from those obtained from GC-FC agar. When the cells were incubated in 50 mM Tris–10 mM EDTA (pH 8.0) for 60 min at room temperature, washed, and then suspended in PBS, no changes in reactivities with any of the agglutinins or lectins were observed. Moreover, when strains of *L. pneumophila* (Knoxville, Togus, Los Angeles, and Chicago) were passaged through weanling guinea pigs (one to five consecutive passages) no modifications (compared with regularly passaged organisms on CYE agar) in agglutinations were found. One strain of *L. pneumophila*, Los Angeles-1 (serogroup 4), failed to react with any of the agglutinins (Table 1). Similarly, *L. bozemanii, L. gormanii*, and *L. micdadii* were found to be nonreactive with the agglutinins or lectins.

The results suggest that the cell surfaces of *Legionellaceae* do not contain carbohydrate groups which are accessible for binding by lectins. Moreover, lectin-reactive carbohydrates were not exposed by incubation of the cells with alkaline Tris-EDTA, a treatment which normally damages the outer membranes of gram-negative bacteria. The agglutinins from *P. americana* (9) and *M. indica* (R. Doyle, unpublished data) do not appear to interact with carbohydrates, but do possess affinities for proteins. The binding specificities of the agglutinins from *A. arborescens* and *A. julibrissin* are also unknown, although the former agglutinin has been reported to precipitate with proteins (4). The surfaces of the *L. pneumophila* strains must be more heterogeneous than reflected by serogrouping, because none of the strains gave identical patterns of agglutination with the plant agglutinins (Table 1). The agglutination patterns may not be directly related to virulence, because the passage of *L. pneumophila* strains through guinea pigs (to increase virulence of the bacteria) did not result in a change in interaction properties with the lectins or agglutinins.

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