Interaction of Lectins with *Yersinia pestis* Strains

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ABSTRACT

The ability of lectins to interact with *Yersinia pestis* strains isolated from rodent fleas and human biological fluids, obtained from different geographic areas, was examined. Lectins of *Canavalia ensiformis, Ulex europaeus, Phaseolus vulgaris,* and *Triticum vulgaris,* as well as a new autochthonous lectin of *Swartzia pickellii* of undefined specificity, were used. Most of the *Y. pestis* strains did not agglutinate with *U. europaeus* or *C. ensiformis* lectin. However, *P. vulgaris* lectin agglutinated suspensions of all the bacillus strains used. Fifteen of the 19 strains tested positive for assays using *S. pickellii* lectin. It is believed this is the first report of *Y. pestis* strain agglutination by lectins. A similar agglutination pattern was obtained for lectins with specificity for oligosaccharides containing *N*-acetylglucosamine and *S. pickellii* lectin, which did bind to the affinity matrix chitin, a polysaccharide of *N*-acetylglucosamine. The use of bacterial strains and commercial lectins of defined specificity may be an approach to providing evidence about the lectin binding sites of undefined monosaccharide specificity.

Index Entries: Lectins; *yersinia pestis.*

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INTRODUCTION

A few attempts have been made to characterize strains of *Yersinia pestis* plague bacillus, isolated from different natural foci found throughout the world (*1–4*). Since previous trials indicated that the bacteria could be agglutinated by lectins, it would be useful to screen *Y. pestis* strains isolated from plague foci existing in different geographic areas of the northeast of Brazil (*5*).

Lectins are remarkable molecules that recognize carbohydrates of glycoconjugates, in solution or bound to membranes (*6*). Plant or animal lectins have been used as valuable probes (*see* reviews by Goldstein and Hayes [*7*] and Lis and Sharon [*8*]) among bacterial differentiation. It has been demonstrated that lectins can define accurately members of the *Neisseriaceae* family (*9*), differentiate species of the genus *Bacillus* (*10*), agglutinate strains of *Neisseria gonorrhoeae* (*11*), and detect intrastrain variations in cell wall carbohydrates or carbohydrate linkages (*12*). Autoagglutination phenomenon has been found to be a drawback, frequently observed in bacteria, and may be a result of partial cell lysis, hydrophobic interactions, salt–salt interactions, or extensive hydrogen bonding (*9*).

A previous trial was made to explore autoagglutination of *Y. pestis* strains under different conditions. The purpose of this study was to verify the potentiality of commercial lectins and a new autochthonous lectin to agglutinate strains of the *Y. pestis* bacillus. To the best of the authors' knowledge, this work represents the first report of *Y. pestis* strain agglutination by lectins.

MATERIALS AND METHODS

Y. pestis Strains

The bacteria were isolated from humans (40 strains), rodents (4 strains), and rodent fleas (2 strains), in plague foci existing in northeastern states of Brazil, from 1966–1982 (*5*), according to the techniques described (*13–17*).

Evaluation of Autoagglutination

The 46 plague strains used were inactivated by formaldehyde and washed 5× in NaCl 150 m*M*. After this treatment, they were separately washed with 50 m*M* sodium phosphate buffer, pH 7.2; 100 m*M* Tris-HCl buffer, pH 7.5 or 100 m*M* Tris-HCl buffer, pH 7.3, containing 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$ and 1 m*M* $MnCl_2$. All buffers contained NaCl 150 m*M*. After each wash, the cells were centrifuged at 1,300×*g*, for 10 min, at room temperature. Cells were suspended at 1% (v/v) in NaCl 150 m*M* or in buffers, at a turbidity that exceeded that of no. 3 MacFarland barium sulfate Standard (*18*). The autoagglutination assay was carried out in microtiter plates

Table 1
Lectins Used in Differentiating *Y. pestis* Strains and Its Specificities

LECTINS	SPECIFICITY		REFERENCES
	MONOSACCHARIDES	OLIGOSACCHARIDES	
Con A	α-D-Man > α-D-Glc > α-D-GlcNAc [a]	Isomal > mal > Suc	7
UEA-I	α-Fuc	Blood group O	7
WGA	β-D-GlcNAc NeuNAc	$(\beta\text{-D-GlcNAc})_3 > (\beta\text{-D-GlcNAc})_2$	7, 19
PHA-E	U [c]	$Gal\beta_4GlcNAc\beta_2Man_6$ $GlcNAc\beta_4 \diagdown Man\beta_4\text{-R}$ [b] $GlcNAc\beta_2Man\alpha_3 \diagup$	20
S. pickellii	U [c]	U [c]	—

[a] Man, mannose; Glc, glucose; Fuc, fucose; Gal, Galactose; GlcNAc, *N*-Acetylglucosamine; NeuNAc, neuraminic acid; Isomal, isomaltose; Mal, maltose; Suc, sucrose.
[b] R = GlcNAcβGlcNAc.
[c] U = undefined.

by adding to each well 50 μL of NaCl 150 m*M* or the above-mentioned buffers, followed by 50 μl of cell suspension. The preparations were left overnight at room temperature before reading.

Commercial Lectins

Lectins from *Canavalia ensiformis* (concanavalin A, Con A), *Triticum vulgaris* (wheat germ agglutinin, WGA), *Phaseolus vulgaris* (PHA-E), and *Ulex europaeus* (UEA-I) were obtained from Sigma Chemical Co., St. Louis, MO, and their specificities are shown in Table 1.

Autochthonous Lectin Preparation

The lectin (isolectins?) from *Swartzia pickellii* Killip (white jacarandá) was a partially purified preparation obtained from legume seeds from which the tegument was removed. A 10% (v/v) extract was made with the seeds homogenized in 150 m*M* sodium phosphate buffer, pH 7.5, containing 150 m*M* NaCl, and kept for 4 h at room temperature. The extract was then submitted to ammonium sulfate factionation. The lectin activity was recovered in the 20–40% fraction (preparation 1), and submitted to ion exchange chromatography. To a DEAE Cellulose column (30.0 × 1.8 cm), containing 76 mL of packed matrix, equilibrated with 10 mM sodium phosphate buffer, pH 7.5, 50 mg of proteins from preparation 1 were-applied. The column was washed with the buffer until A_{280nm} was 0.03.

The elution was performed with a step gradient of 100 and 200 m*M* NaCl in the phosphate buffer, 2× the column vol for each step. Two-mL fractions were collected at a flow rate of 20 mL/h. The lectin was recovered in the buffer containing 200 mM NaCl (preparation 2). The activity was assayed using human erytrocytes of AB blood group (*21*), treated with glutaraldehyde (*22*).

Agglutination Assays

To microtiter plates, 50 μL of 150 m*M* NaCl were placed in the wells, and 50 μL of lectin preparation (1 mg/mL) in 150 m*M* NaCl were added, to obtain serial dilutions. Then 50 μL of cell suspension in 150 mM NaCl, prepared as for autoagglutination evaluation, were added to each well. After resuspension, the material was left overnight at room temperature and read for agglutination activity.

RESULTS

Autoagglutination of *Y. pestis* Strains

An intense autoagglutination was detected when 46 formalinized strains of *Y. pestis* were washed in saline or buffers. The positive autoagglutination pattern of these strains was as follows: 19 in 150 m*M* sodium chloride, 28 in Tris-HCl 100 m*M* buffer, pH 7.5, containing 150 m*M* sodium chloride, 36 in 50 m*M* sodium phosphate buffer, pH 7.2, containing 150 m*M* sodium chloride, and 37 in 100 m*M* Tris-HCl buffer, pH 7.3, containing 150 m*M* sodium chloride and 1 m*M* of $CaCl_2$, $MgCl_2$, and $MnCl_2$. Only 7 strains were nonautoagglutinated in the above-described conditions. Therefore, 150 m*M* sodium chloride was selected for the agglutination assay.

S. pickellii Lectin Preparation

S. pickellii lectin activity was not inhibited by 30 different carbohydrates used. Approximately 70% of the lectin activity in the extract was recovered in preparation 1, and when preparation 1 was applied to DEAE Cellulose, 20% of the activity was obtained in preparation 2. This material, as shown by polyacrylamide electrophoresis for native proteins, consisted of a major protein band, 3 secondary and a very faint band, with isoelectric pH values from 3.8–4.5 (results not shown). A similar pattern was obtained when preparation 1 was biospecifically adsorbed to the affinity matrix chitin and desorbed nonbiospecifically with 10 m*M* sodium phosphate buffer, pH 6.5, containing 0.25, 0.5, or 1.0 *M* NaCl, or when preparation 2 was rechromatographed in DEAE Sephadex A-50.

Table 2
Identification of *Y. pestis* Strains by Lectins

STRAINS		LECTINS				
Identification [a]	Origin	UEA-I	Con A	PHA-E	WGA	*S. pickellii*
Exu (PE) /188/1967	human	–	–	+	+	–
Exu (PE) /210/1968	human	–	–	+	+	+
Exu (PE) /247/1968	human	–	–	+	+	+
Guaraciaba do Norte (CE) / 509/1971	human	–	–	+	+	+
Ipu (CE) /543/1971	human	–	–	+	+	+
Ipubi (PE) /845/1975	human	–	–	+	+	–
Araripina (PE) /783/1975	human	+	–	+	+	+
Guaraciaba do Norte (CE) / 789/1978	human	–	–	+	+	+
Aratuba (CE) /795/1978	human	–	–	+	+	+
Ipu (CE) /797/1978	rodent	+	+	+	+	+
São Benedito (CE) /803/1978	human	–	+	+	+	+
Capistrano (CE) /805/1978	human	–	–	+	+	+
Ipu (CE) /809/1979	human	–	–	+	+	+
São Caetano (PE) /826/1979	rodent flea	–	–	+	+	+
Ipu (CE) /835/1979	rodent	–	–	+	+	+
Ipu (CE) /743/1979	rodent	–	–	+	+	+
Rubelita (MG) /01/1983	rodent	–	–	+	+	+
Bahia (BA) /02/1984	rodent flea	–	–	+	+	+
Poções (PE) /03/1984	human	–	–	+	+	+

[a]Plague Laboratory Collection from Aggeu Magalhaes Research Center PE. The identification is made on the basis of the city of plague focus, catalog number, and year of isolation.

Interaction Between *Y. pestis* Strains and Lectins

When suspensions of 19 selected strains were tested with lectins, a different pattern of agglutination was observed (Table 2). Only 1 strain did agglutinate with all lectins used. However, all strains were positive when PHA-E or WGA were used. The preparation from *S. pickellii* lectins did agglutinate most to the used strains. The origin of the strains did not seem to interfere with the lectin agglutination patterns obtained.

DISCUSSION

Several procedures have proven successful to minimize or abolish autoagglutination in bacteria used in agglutination assays with lectins (*9,11,23*). *Y. pestis* strains did show a high incidence of autoagglutination. No efforts were made to minimize this phenomenon, since inactivated strains were used.

Two of the 19 *Y. pestis* strains used did agglutinate with Con A, a glucose/mannose lectin, and with UEA-I, specific for fucose. In fact, fucose was detected in a lipopolysaccharide isolated from the virulence factor F1 of *Y. pestis* (*24*). The results also suggested that *Y. pestis* strains were homogeneous in relation to the presence of *N*-acetylglucosaminyl residues, since they all appeared to have these residues available in their cell wall. A polysaccharide associated with the virulence factor F1 of *Y. pestis*, consisting predominantly of *N*-acetylglucosamine and hexuronic acid was mentioned by Bakharkh (*25*). The lectins showed potential for use in the differentiation of *Y. pestis* strains. A broader investigation might demonstrate the application of lectins for epidemiological studies.

The family of proteins obtained from *S. pickellii* by conventional chromatography or by affinity to chitin, under different conditions, had very similar properties. The *S. pickellii* lectin behaved similarly to PHA-E and WGA, which are effectively inhibited by oligosaccharides containing *N*-acetylglucosamine. In addition, *S. pickellii* lectin did bind to chitin, a polysaccharide of *N*-acetylglucosamine. The use of a panel of lectins of defined specificities and a set of bacterial strains differentiated by these versatile proteins can help to unravel the nature of the binding sites from new lectins of undefined specificities.

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