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Differentiation of *Bacillus anthracis* and Other *Bacillus* Species by Lectins

HUGH B. COLE,[1] JOHN W. EZZELL, JR.,[2] KENNETH F. KELLER,[1] AND RONALD J. DOYLE[1]*

Department of Microbiology and Immunology, University of Louisville Health Sciences Center, Louisville, Kentucky 40292,[1] and Division of Bacteriology, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21701[2]

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Bacillus anthracis was agglutinated by several lectins, including those from *Griffonia simplicifolia*, *Glycine max*, *Abrus precatorius*, and *Ricinus communis*. Some strains of *Bacillus cereus* var. *mycoides* (*B. mycoides*) were strongly reactive with the lectin from *Helix pomatia* and weakly reactive with the *G. max* lectin. The differential interactions between *Bacillus* species and lectins afforded a means of distinguishing *B. anthracis* from other bacilli. *B. cereus* strains exhibited heterogeneity with respect to agglutination patterns by lectins but could readily be differentiated from *B. anthracis* and the related *B. mycoides*. Spores of *B. anthracis* and *B. mycoides* retained lectin receptors, although the heating of spores or vegetative cells at 100°C resulted in a decrease in their ability to be specifically agglutinated. Fluorescein-conjugated lectin of *G. max* stained vegetative cells of *B. anthracis* uniformly, suggesting that the distribution of lectin receptors was continuous over the entire cellular surface. *B. anthracis* cells grown under conditions to promote the production of capsular poly(D-glutamyl peptide) were also readily agglutinated by the lectins, suggesting that the lectin reactive sites penetrate the polypeptide layer. Trypsin, subtilisin, lysozyme, and mutanolysin did not modify the reactivity of *B. anthracis* with the *G. max* agglutinin, although the same enzymes markedly diminished the interaction between the lectin and *B. mycoides*. Because the lectins which interact with *B. anthracis* are specific for α-D-galactose or 2-acetamido-2-deoxy-α-D-galactose residues, it is likely that the bacteria possess cell surface polymers which contain these sugars. Lectins may prove useful in the laboratory identification of *B. anthracis* and possibly other pathogenic *Bacillus* species, such as *B. cereus*.

Most species of the genus *Bacillus* are saprophytic and are widely distributed in nature, particularly in soils. One organism, *Bacillus anthracis*, is an important pathogen in humans and cattle and may lead to a serious disease called anthrax. Workers at clinical laboratories are presented with many problems when attempting to identify *B. anthracis* from a specimen (12, 19). The laboratory identification of members of the genus *Bacillus* may involve biochemical reactions, immunofluorescence, bacteriophage typing, production of capsule, analysis of composition of lipids, and determination of nucleic acid homologies (reviewed in reference 1). There are close relationships between *B. anthracis*, *Bacillus cereus*, *Bacillus mycoides*, and *Bacillus thuringiensis* in terms of antigenic structures of surface components (7, 13, 18, 20, 21, 23), metabolism (16, 18, 24, 25), and DNA-DNA homologies (17, 31, 33, 35). Serological methods have generally been unsuccessful in identifying *B. anthracis* (7, 12, 13, 18). Moreover, bacteriophage typing (5) is not absolutely specific, as other bacilli may adsorb *B. anthracis* bacteriophage (2, 3, 7). Studies have concluded that there is no single criterion, including pathogenicity, that separates *B. anthracis*, *B. cereus*, *B. mycoides*, and *B. thuringiensis* (28).

We have noted that lectins are convenient reagents for the study of cell surfaces of bacilli (6, 8, 10, 32). The glucosylated cell wall teichoic acid of *Bacillus subtilis* 168 can be purified by using affinity chromatography on concanavalin A (ConA)-Sepharose columns (9). Furthermore, the distribution of glucosylated cell wall teichoic acids on the *B. subtilis* cell surface can be monitored by use of fluorescent ConA (10). Because *B. anthracis* is known to possess a galactose-containing polysaccharide on its cell envelope (4, 26), it was reasoned that galactose-binding lectins may be agents which could selectively agglutinate the bacterium. In this report, we describe procedures which enable the rapid differentiation of *B. anthracis* from other bacilli. The methods employ galactose-binding lectins and can be completed within a few minutes.

* Corresponding author.

MATERIALS AND METHODS

Reagents and chemicals. All lectins and agglutinins, including fluorescein-labeled soybean agglutinin, were supplied by E-Y Laboratories, San Mateo, Calif. (Table 1). The lectins were affinity purified, except for SRA (from *Sarothamnus scoparius*), which was an ammonium sulfate precipitate. Calcium chloride was obtained from J. T. Baker Chemical Co., Phillipsburg, N.J. Reagent manganous chloride and urea were obtained from Fisher Scientific Co., Fairlawn, N.J. Reagent-grade sucrose, sodium dodecyl sulfate, trypsin, subtilisin, succinic anhydride, and lysozyme were products of Sigma Chemical Co., St. Louis, Mo. Complex media were obtained from Difco Laboratories, Detroit, Mich., or from BBL Microbiology Systems, Cockeysville, Md. Mutanolysin (38) was a gift from K. Yokagawa, Dainippon, Ltd., Osaka, Japan.

Organisms and culture conditions. Sources of strains of *Bacillus* species used are listed in Table 2. All strains were maintained on AK sporulation agar (BBL), except for *Bacillus globisporus*, which was maintained on tryptose blood agar base (Difco). Cells and spores were stored at 4°C before transfer to new slants or media. For agglutination assays, most cells were obtained from overnight growth at 37°C on tryptose blood agar base plates, whereas *B. globisporus* and *B. mycoides* were cultured at room temperature before being harvested. Cells were recovered with a wetted cotton swab

TABLE 1. Lectins used to agglutinate *Bacillus* species[a]

Lectin	Specificity[b]
Abrus precatorius (APA)	β-D-Gal > α-D-Gal
Arachis hypogeae (PNA)	D-Gal-β-(1 → 3) > β-D-GalNH$_2$ = α-D-Gal
Bauhinia purpurea (BPA)	D-GalNAc > D-Gal
Canavalia ensiformis (ConA)	α-D-Man > α-D-Glc > α-D-GlcNAc
Dolichos biflorus (DBA)	α-D-GalNAc > α-D-Gal
Glycine max (SBA)	α-D-GalNAc ≥ β-D-GalNAc ≫ α-D-Gal
Griffonia simplicifolia (GSA-I)	α-D-Gal > α-D-GalNAc
Griffonia simplicifolia (GSA-II)	α-D-GlcNAc = β-D-GlcNAc
Helix aspersa (HAA)	α-D-GalNAc = α-D-GlcNAc
Helix pomatia (HPA)	α-D-GalNAc > α-D-GlcNAc ≫ α-D-Gal
Limulus polyphemus (LPA)	sialic acid
Lotus tetragonolobus (Lotus A)	α-L-Fuc = 2-*O*-Me-D-Fuc
Maclura pomifera (MPA)	α-D-Gal = α-D-GalNAc
Phaseolus limensis (LBA)	α-D-GalNAc > α-D-Gal
Phaseolus vulgaris (PHA-E)	D-GalNAc
Pisum sativum (PEA)	α-D-Man > α-D-Glc > α-D-GlcNAc
Ricinus communis (RCA-I)	β-D-Gal > α-D-Gal
Ricinus communis (RCA-II)	β-D-Gal > β-D-GalNAc
Robinia pseudoacacia (RPA)	unknown (possibly sialoglycopeptides)
Sarothamnus scoparius (SRA)	α-D-Gal > α-L-Fuc
Solanium tuberosum (STA)	(β-D-GlcNAc)$_{2-5}$ > β-D-GlcNAc
Sophora japonica (SJA)	β-D-GalNAc > β-D-Gal
Triticum vulgarius (WGA)	(β-D-GlcNAc)$_3$ > (β-D-GlcNAc)$_2$ > β-D-GlcNAc
Ulex europaeus (UEA-I)	α-L-Fuc
Ulex europaeus (UEA-II)	(β-D-GlcNAc)$_2$ > β-D-GlcNAc

[a] Specificities of all lectins were obtained from E · Y Laboratories or from Goldstein and Hayes (14).
[b] Gal, Galactose; GalNAc, *N*-acetylgalactosamine; Man, mannose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Fuc, fucose; 2-*O*-Me-D-Fuc, 2-*O*-methylfucose.

and suspended in phosphate-buffered saline (PBS) (40 mM sodium phosphate, 150 mM sodium chloride, 0.1 mg of sodium azide per ml [pH 7.3]).

Spore growth and preparation. Sporulation was accomplished by a modification of the method used by Eisenstadt and Silver (11). Inocula were taken from tryptose blood agar base plates and suspended in tryptic soy broth (Difco) supplemented with 100 μM calcium chloride and 10 μM manganous chloride. Cells were vigorously shaken for 64 h at 37°C. Spores were washed twice in PBS and then further purified by sedimenting twice in 55% sucrose. The enriched spores were then suspended in PBS to an optical density of 0.6 ± 0.1 at 450 nm (1-cm path length) and incubated with mutanolysin (50 μg/ml final concentration in PBS) or lysozyme (50 μg/ml final concentration in PBS) for 17 ± 2 h at 37°C. Spores were then washed twice by centrifugation and suspension in PBS. Preparations were examined with Gram stain and by phase-contrast microscopy for rod-shaped cells. Only spore preparations judged to be free of intact cells were used in agglutination assays.

Agglutination test procedures. Procedures for agglutination were adapted from the methods used by Schaefer et al. (32) for the genus *Neisseria*. Both vegetative cell and spore suspensions were tested in the same manner. Agglutination tests were carried out on Boerner microtiter plates (Curtin Matheson Scientific, Inc., Cincinnati, Ohio). Lectins were diluted in PBS to a concentration of 200 μg/ml and stored at 4°C. Test wells were set up opposite to control wells for direct test-control comparisons. In each test well, 50 μl of cell suspension was added to 50 μl of lectin. In one control well, 50 μl of buffer was added to 50 μl of lectin to detect any false-positives due to a precipitation reaction between lectin and buffer. In the other control well, 50 μl of cell suspension was mixed with 50 μl of buffer. Plates were then shaken on a Tektator V rotary shaker for 10 min at 150 rpm. It was important not to permit the cells to incubate with the lectins for extended time periods, e.g., >1 h, because a loss of specificity was observed. Plates were examined for evidence of agglutination reaction under an Olympus VMT stereo microscope. Occasionally, cells exhibited autoagglutination

TABLE 2. Sources of bacteria used

Bacillus species	Source
B. anthracis 11966, 14185; *B. cereus* 6464, 7064, 19637, 11778, E14579, 23260, 13472, 246; *B. mycoides* 6462; *B. lentus* 10840; *B. globisporus* 23301	American Type Culture Collection, Rockville, Md.
B. cereus, *B. mycoides*, *B. brevis*, *B. megaterium*, *B. licheniformis*, *B. circulans*, *B. pumilus*	Midwest Culture Service, Terre Haute, Ind.
B. anthracis V-770, ATCC 4229, Colorado, KAN7322, S. Africa 205, M36, Texas, Ames, Vollum 1B, Sterne; *B. cereus* T, 4915, 9620, 9634; *B. mycoides* USAMRIID; *B. thuringiensis* 4040, 4041, 4042-b, 4045, 4055, 4065	U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, Md. (culture collection)
B. sphaericus 1593	Bacillus Genetic Stock Center, Columbus, Ohio
B. anthracis 1103	University of Michigan Research Laboratory, Ann Arbor, Mich.
B. amyloliquefaciens N	M. Courtney, University of Rochester, N.Y.
B. megaterium KM *ade* Prt$^-$	S. Graham, University of Louisville, Ky.
B. circulans 14175, 14176, 9500, 11033, 7049, 4513; *B. polymyxa*; *B. coagulans*	R. E. Gordon, Waksman Institute, Piscataway, N.J.

in PBS. In most cases, these autoagglutinations were not significant enough to bias the lectin agglutination readings.

Fluorescein labeling of cells and spores. Fluorescein-labeled SBA (400 μg/ml in PBS) was mixed with an equal volume of cells (usually 100 μl) or spores. The suspensions were incubated at room temperature for 10 to 15 min with gentle shaking. The suspensions were then washed twice in PBS to remove unbound lectin. Samples were finally dried on microscope slides. Specimens were observed by fluorescence microscopy (Carl Zeiss, Inc., New York, N.Y.). Photographs were taken with a Nikon FM with a Nikomat model 2 microscope adapter (Nikon Inc., Garden City, N.Y.), using Kodak ASA 400 color print film (Eastman Kodak Co., Rochester, N.Y.).

Modification of cell surface structures. *B. anthracis* ATCC 11966 and *B. mycoides* ATCC 6462 were subjected to enzymic and chemical modifications. Cells were washed twice in PBS and suspended in the buffer to an optical density of 0.5. Succinic anhydride (5.0 mg/ml in acetonitrile) was added to a final concentration of 100 μg/ml. The suspension was then incubated for 2 h at room temperature, after which the cells were washed twice and suspended in PBS. Enzyme treatments involved incubating the washed cells in 50 μg/ml final concentrations of either trypsin, mutanolysin, subtilisin, or lysozyme at 37°C for 2 h. The cells were then washed twice and suspended in PBS. Some cell preparations were treated with sodium dodecyl sulfate or concentrated urea. These cells were washed three times in PBS and suspended in the buffer. All modified cell suspensions were then used in agglutination assays.

RESULTS

Interaction between *Bacillus* species and lectins. To compare agglutination patterns, the *Bacillus* species were arbitrarily placed into Analytab Products Inc. (API) groups. The API groupings for *Bacillus* species depend on metabolic activities (24, 25) and are useful in establishing taxonomic relationships between closely related species and in establishing simple methods for their identification from clinical specimens. Group I included *B. anthracis*, *B. cereus*, *B. mycoides*, and *B. thuringiensis* (24). Table 3 shows interaction of the bacilli suspensions with purified lectins (see Table 1 for a description of lectins). All strains of *B. anthracis* listed in Table 2 were agglutinated by lectins RCA-I, RCA-II, APA, GSA-I, and SBA. Similar reactivities were exhibited by *B. mycoides*, but these species were also agglutinated by HPA. The lectins which agglutinated *B. anthracis* and *B. mycoides* were capable of interacting with D-galactose (D-Gal) or 2-acetamido-2-deoxy-D-galactose (*N*-acetylglucosamine [GalNAc]) (Table 1). Some lectins, however, with similar carbohydrate-binding specificities, were incapable of agglutinating the bacilli (Table 3).

For *B. cereus*, great heterogeneity was observed in terms of interactions with the lectins (Table 3). Several *B. cereus* strains were agglutinated by SBA or APA. *B. thuringiensis* strains were also generally refractory to lectins. This is significant since *B. thuringiensis* is generally difficult to differentiate from *B. anthracis*. Lectins which failed to agglutinate any of the bacilli included GSA-II, PNA, PEA, MPA, DBA, PHA-E, HAA, SJA, UEA-I, UEA-II, RPA, Lotus A, and LBA (Table 1).

Representative species of other *Bacillus* API groups were found not to readily agglutinate with lectins. Only *Bacillus sphaericus*, *B. subtilis* 168, and *Bacillus amyloliquefaciens* were agglutinable with ConA. The cell receptor probably responsible for interaction with ConA was α-D-glucosylated teichoic acid (8–10). Weak agglutination of *B. subtilis* strains 168 and W23 by LPA was observed, possibly due to specific interaction between the lectin and glycerol or ribitol teichoic acids (30).

***Bacillus* spores and lectins.** Members of the genus *Bacillus* can undergo metabolic changes leading to the formation of endospores. The spores are generally considered to possess internal cell wall components surrounded by multiple coats of protein (22). During the vegetative cell-to-spore transition, considerable surface modification must occur, but it is unknown whether the spores retain lectin-reactive sites or even whether there are new and different sites synthesized. Lectin agglutination tests for *B. anthracis* and other bacilli would be greatly strengthened if the spores retained their lectin receptors. We purified spores of several *Bacillus* species by density centrifugation in sucrose and by digestion of intact cells with lysozyme and mutanolysin (38) (in other experiments we have found that mutanolysin is a useful enzyme for the dissolution of walls of API group I bacilli; G. Zipperle, J. Ezzell, and R. J. Doyle, submitted for publication). Purified spores were then mixed with lectins (Table 4). The results provide evidence to suggest that spores of *B. anthracis* and *B. mycoides* can also be distinguished by lectins. In fact, spores and vegetative cells of both of these

TABLE 3. Interactions between lectins and API group I *Bacillus* species[a]

Organism	APA	GSA-I	RCA-I	RCA-II	SBA	ConA	WGA	BPA	HPA	SRA	LPA
B. anthracis 11966	+	+	+	+	+	−	−	−	−	−	−
B. anthracis 14185	+	+	+	+	+	−	−	−	−	w	−
B. anthracis 4229	+	+	+	+	+	−	+	−	−	−	−
B. cereus 4915	−	−	−	−	−	−	+	−	−	−	−
B. cereus 11778	−	+	−	−	−	−	+	−	+	−	−
B. cereus E14578	−	+	−	−	−	−	−	−	+	−	−
B. cereus 246	−	−	−	−	−	−	−	−	+	−	−
B. cereus T	−	−	−	−	−	−	−	−	w	−	w
B. cereus 7064	−	+	+	+	−	−	−	−	−	−	−
B. cereus 23260	−	−	−	−	−	−	−	−	w	−	−
B. cereus 19637	−	−	−	−	−	−	−	−	−	−	−
B. mycoides MWC	+	+	+	+	w	−	−	−	+	−	−
B. mycoides USAMRIID	+	+	+	+	w	−	−	−	+	−	−
B. mycoides 6462	+	+	+	−	+	−	−	−	+	−	−
B. thuringiensis 4040	−	−	−	−	−	−	−	−	−	−	−

[a] Agglutinations were scored as + (positive), − (negative), or w (weak).

TABLE 4. *Bacillus* spores and lectin agglutination tests

Spores	LECTIN													
	APA	GSA-I	RCA-I	RCA-II	SBA	ConA	WGA	HPA	SRA	RPA	HAA	GSA-II	UEA-II	MPA
B. anthracis 11966	+	+	+	+	+	−	−	−	−	−	−	−	−	−
B. anthracis 14185	+	+	+	+	+	−	−	−	−	−	−	−	−	−
B. cereus T	−	−	−	−	−	−	w	+	−	−	w	w	−	−
B. cereus 6464	+	−	−	−	−	−	−	+	−	−	w	−	−	−
B. cereus 9634	−	−	−	−	−	−	−	+	−	−	w	−	−	−
B. cereus 23260	−	−	−	−	−	−	−	−	w	w	w	−	+	+
B. cereus E14579	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B. cereus 19637	−	−	−	−	−	+	−	+	−	−	+	−	−	−
B. cereus 246	−	−	−	−	−	w	−	−	−	−	−	−	−	−
B. mycoides 6462	w	−	w	w	−	−	−	+	−	−	−	−	−	−
B. mycoides MWC	w	w	w	w	+	−	−	+	−	−	−	−	−	−
B. subtilis 168	−	−	−	−	−	+	−	−	−	−	−	−	−	−

species appear to be agglutinated by the same lectins (Table 3). The HAA lectin was able to weakly agglutinate several spores from *B. cereus* strains but not the respective vegetative cells. Furthermore, MPA, UEA-II, and ConA were able to agglutinate some spores but no vegetative cells. *B. subtilis* 168 vegetative cells and spores were agglutinated by ConA. The results support the view that lectins can also be used as selective agglutinating reagents for bacterial spores.

Vegetative cells and spores of several strains of *B. anthracis* and *B. mycoides* were titrated with SBA, GSA-I, and HPA. It was found that, in general, *B. anthracis* vegetative cells could more readily bind SBA than *B. mycoides* cells (Table 5). When the agglutinations of spores were compared to vegetative cells, it was observed that the spores tended to interact less strongly with lectins. When either cells or spores were heated to 100°C for 15 min and cooled, a higher concentration of lectin was usually required to elicit agglutination. The heating of cells or spores apparently results in loss or modification of lectin binding sites.

Distribution of lectin binding sites on *B. anthracis*. In previous studies, it was shown that fluorescein-labeled ConA bound over the entire surface of *B. subtilis*, although the lectin may have been more concentrated at septa (10). Lectin receptor sites may possibly be found on cell poles, septa, and cylinders of bacilli. Washed cells and spores of *B. anthracis* ATCC 11966 were interacted with fluorescein-labeled SBA and examined by fluorescence microscopy. The results (Fig. 1 and 2) reveal that the lectin tends to bind evenly over all parts of the vegetative cells. Moreover, the results also confirm the observation that spores of *B. anthracis* interact with the lectin.

TABLE 5. Concentrations of lectins required for agglutination of vegetative cells and spores

Organism	Concn of lectin (μg/ml)[a]		
	SBA	GSA-I	HPA
B. anthracis 14185	12.5 (neg)	Neg (neg)	Neg (neg)
B. anthracis 11969	6.3 (50)	6.3 (50)	Neg (neg)
B. anthracis MWC	3.1 (12.5)	3.1 (25)	Neg (neg)
B. anthracis 14185 (spores)	25 (50)	Neg (neg)	Neg (neg)
B. anthracis 11969 (spores)	25 (50)	Neg (neg)	Neg (neg)
B. mycoides 6462	100 (neg)	100 (neg)	3.1 (12.5)
B. mycoides MWC	25 (50)	12.5 (neg)	6.3 (50)
B. mycoides USAMRIID	12.5 (50)	3.1 (3.1)	6.3 (6.3)
B. mycoides 6462 (spores)	Neg (neg)	Neg (neg)	25 (50)

[a] Values shown represent minimal concentrations of lectins required to elicit a positive agglutination reaction. Numbers in parentheses are results obtained after boiling cells or spores in PBS for 15 min. Neg, No detectable agglutination.

Removal of lectin receptors from *B. anthracis* and *B. mycoides*. *B. anthracis* and *B. mycoides* were subjected to several kinds of extractions or enzyme treatments to modify lectin receptor sites such that one organism may be more readily differentiated from the other by either SBA or HPA. The cells were treated with protein extractants (0.1% sodium dodecyl sulfate) and proteases (trypsin and subtilisin). If lectin receptors were removed or modified by the treatments, then the amounts of lectins required for agglutination may be changed. The results (Table 6) show that lysozyme, mutanolysin, trypsin, and subtilisin destroyed or weakened the agglutinability of *B. mycoides* ATCC 6462 by SBA, whereas HPA receptors remained intact. In addition, 8 M urea was also effective in rendering *B. mycoides* insensitive to SBA. In contrast, treatment of *B. anthracis* by the same enzymes or extractants did not greatly modify reactivity with either SBA or HPA. One reagent, succinic anhydride, designed to increase the overall negative surface charge, did not alter the binding of either *B. anthracis* or *B. mycoides* with the two lectins. Overall, the results appear to reveal that the surface of *B. mycoides* is less resistant than *B. anthracis* to chemical or protease challenge.

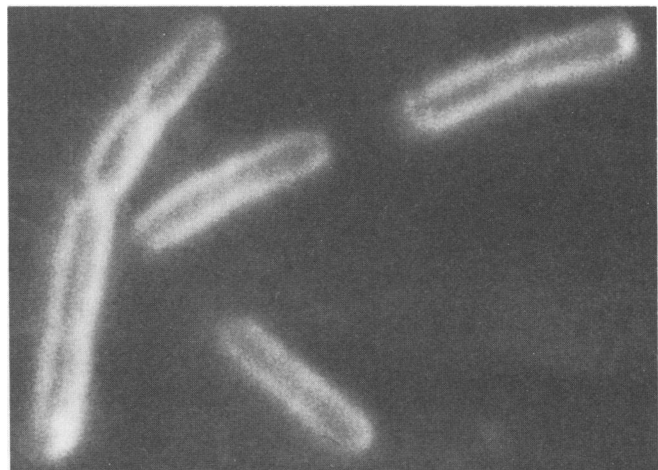

FIG. 1. Binding of fluorescein-conjugated SBA to *B. anthracis* 11966.

DISCUSSION

Several factors may be involved in the interaction between bacterial cell surfaces and lectins. Not only must an organism possess the proper carbohydrate determinants on its surface, but other factors such as lectin molecular weight, hydrophobic group stabilization, hydrogen ion concentration, and ionic strength are also important.

When *Bacillus* species were interacted with purified lectins of differing specificities, it was observed that several of the proteins could agglutinate *B. anthracis* and *B. mycoides* strains. These lectins were generally of a specificity for D-Gal or GalNAc and included SBA, GSA-I, RCA-I and RCA-II, and APA (Table 3). Another lectin, HPA, also specific for GalNAc and D-Gal, agglutinated *B. mycoides* but not *B. anthracis*, thereby affording a means of distinguishing the two species. It is surprising that lectins such as BPA, MPA, HAA, LBA, and others, although readily reactive with Gal or GalNAc groups (14), would agglutinate neither *B. anthracis* nor *B. mycoides*. The results suggest a rapid means of identifying *B. anthracis* from a colony or pure culture. Agglutination by SBA, the nontoxic soybean agglutinin, identifies the cells as either *B. anthracis* or *B. mycoides*, and the HPA lectin specifically agglutinates the latter bacterium. Moreover, spores can also be identified by the same means (Table 4). The lectin agglutination tests therefore constitute a considerable advance in technology for the identification of *B. anthracis* cultures obtained from clinical specimens.

The composition of the polymer(s) or cell surface components(s) responsible for interacting with the lectins is unknown. Mester et al. reported that *B. anthracis* possessed a polymer composed of Gal, acetylated Gal, and 2-amino-2-deoxy-D-glucose (26). This polymer was poorly immunogenic in rabbits (4, 15) and may not be a prominent surface antigen of the organism. The diagnostic value of the polymer may have therefore been overlooked. It is possible that the reactive lectins were able to interact with this polymer in *B. anthracis* and its close taxonomic species, *B. mycoides*. Because the molecular weight of SBA is 120,000 (14) and the molecular weight of HPA is 26,000 (14), it is assumed that the tertiary structures of the lectins govern their ability to bind to potential receptors on cell surfaces. Steric factors may also be involved in the inhibition of γ-phage binding to *B. anthracis* by WGA (37), even though WGA does not agglutinate the bacteria (Table 3).

The fact that spores retained lectin binding sites can possibly be explained. The spores may have retained the lectin receptors in an unmodified form, and the receptors could have penetrated the spore coats or could have been

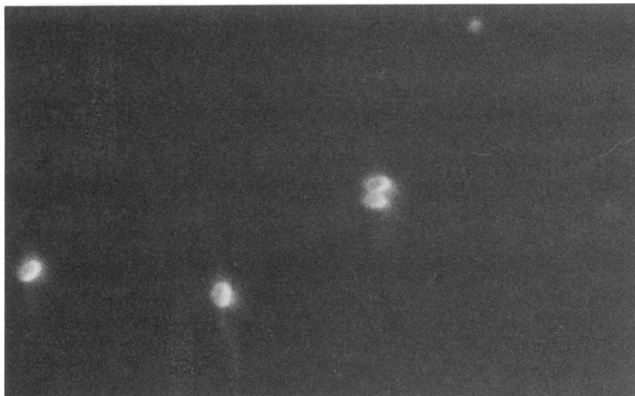

FIG. 2. Interaction between spores of *B. anthracis* 11966 and fluorescein-conjugated SBA.

TABLE 6. Chemical and enzymatic modification of the interaction between lectins and *B. anthracis* and *B. mycoides*[a]

Treatment	Minimal lectin concn (μg/ml) for			
	B. anthracis agglutination with		*B. mycoides* agglutination with	
	SBA	HPA	SBA	HPA
None, control	6.25	Neg	100	1.6
0.1% SDS[b], 100°, 30 min	3.1	Neg	100	3.1
8 M urea, 2 h	3.1	Neg	Neg	3.1
Succinic anhydride	12.5	Neg	100	3.1
Trypsin	6.3	Neg	Neg	3.1
Subtilisin	3.1	Neg	Neg	3.1
Lysozyme	6.25	Neg	200	3.1
Mutanolysin	12.5	Neg	Neg	3.1

[a] Values shown are the minimal concentrations of lectins required for detectable agglutination. Neg, No detectable agglutination.
[b] SDS, Sodium dodecyl sulfate.

components of the spore coats. Conversely, the spores may contain completely different lectin receptors, but of similar composition, and were therefore capable of interacting with the lectins. Support for this view comes from the observation that several *B. cereus* strains expressed different lectin receptors on spores and vegetative cells (Tables 3 and 4). For example, cells of *B. cereus* T were refractory to agglutination by WGA, HAA, and GSA-II, whereas spores were agglutinated by these lectins. It is known that spores and vegetative cells of several *Bacillus* species possess common antigens (27, 29). These antigens may, in certain cases, be the lectin receptors. Finally, it must also be considered that the spores were not completely freed of vegetative cells or cell structures.

The results also reveal the heterogeneity of *B. cereus* strains. A lectin specific for *N*-acetylglucosamine, WGA, agglutinated only *B. cereus* strains 4915 and 11778 (Table 3). The lectin GSA-I, specific for Gal and GalNAc residues, agglutinated only *B. cereus* strains 11778, E14578, and 7064. *B. cereus* T was agglutinated by LPA. A general pattern of reactivity was not found for *B. cereus*, although the results clearly distinguish *B. cereus* from *B. anthracis* and *B. mycoides*. It would be interesting to examine the lectin agglutination reactions of *B. cereus* strains obtained from eye infections (34) and food poisoning (36).

When cells or spores were boiled in PBS before interaction with lectin, it was found that more lectin was usually required to elicit agglutination (Table 5). These results suggest that the heat treatment may have extracted some of the lectin receptors. Another explanation is that heat treatment changed the conformation or distribution of the receptors, although this does not appear likely. The observations that proteases and chaotropic agents do not markedly modify reactivity of *B. anthracis* with SBA suggest that the lectin-binding sites on the cells are not protein, nor are they necessarily associated with surface protein. The receptors must contain Gal or GalNAc, but it is unlikely that these carbohydrates are covalently bound to protein since glycoproteins in bacteria are rare. The loss of agglutinability by SBA when *B. mycoides* was treated with heat, detergents, or enzymes may suggest that the SBA receptors were removed or extracted. In contrast, the retention of HPA receptors by *B. mycoides* after the same treatments suggests that the HPA receptors and the SBA receptors are distinct molecules, although both receptors probably contain D-Gal or D-GalNAc.

We believe that lectins may have importance in the clinical laboratory identification and possible taxonomic classification of *Bacillus* species. The results of this paper provide evidence which shows that *B. anthracis* and *B. mycoides* can be distinguished from each other and from other bacilli by only two lectins. Because lectins are monoclonal proteins and because they possess a spectrum of specificities and molecular weights, it is to be expected that they will provide substantial tools for diagnostic microbiology studies.

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