ANTIGENIC RELATIONSHIP OF THE ENDOSPORES OF *BACILLUS CEREUS*-LIKE INSECT PATHOGENS TO *BACILLUS CEREUS* AND *BACILLUS ANTHRACIS*[1]

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Aerobic sporeforming bacilli pathogenic for insects are known. In their appearance and cultural characteristics they are much like *Bacillus cereus*, except for their habit of depositing during sporulation crystals of protein known as parasporal bodies. Heimpel and Angus (1958) have reviewed the taxonomic status of these crystalliferous spore bearing bacilli. The present study has had as its purpose the revelation of the possible existence of agglutinogenic relationships between the endospores of the insect pathogenic bacilli and the endospores of *B. cereus* and *Bacillus anthracis*. Since agglutinin cross reactions have been demonstrated between the spores of *B. cereus* and *B. anthracis* (Lamanna and Eisler, 1960), it is of interest to know whether these cross reactions extend to the insect pathogenic sporeforming organisms.

MATERIAL AND METHODS

Strains. Dr. E. A. Steinhaus kindly supplied the following representative strains of the insect pathogenic crystalliferous spore bearers: 0-3-30, *Bacillus thuringiensis* var. *thuringiensis* Berliner; 16-2-2, *Bacillus thuringiensis* var. *thuringiensis* Berliner; 0-24-3, *Bacillus thuringiensis* var. *alesti* Toumanoff and Vago; 0-32-2, *Bacillus thuringiensis* var. *alesti* (Anduze strain); 0-28-1, *Bacillus thuringiensis* var. *sotto* Aoki and Chigasaki; 57-1-1 *Bacillus entomocidus* var. *entomocidus* Heimpel and Angus; and 58-1-1 *Bacillus entomocidus* var. *subtoxicus* Heimpel and Angus.

The origins of the *B. cereus* and *B. anthracis* strains employed are listed in the paper by Lamanna and Eisler (1960).

Preparation of spore antigens and agglutination studies. With only minor modifications, preparation of spore antisera free of vegetative bacilli agglutinins and agglutination tests were performed as outlined by Lamanna and Eisler (1960). The one notable change was the employment as diluent for the agglutination tests of 0.85% NaCl solution with 0.5% bovine serum albumin added. The presence of the albumin was found to improve the appearance of serological agglutination of spores, and to reduce both the degree of spontaneous agglutination and the tendency of spores to be trapped in a meniscus.

RESULTS AND DISCUSSION

Antisera prepared against the endospores of the insect pathogens were used for agglutination tests with the results recorded in Table 1. Cross reactions occur among the insect pathogenic strains and between these organisms and *B. cereus* and *B. anthracis*. That the antigenic relationship between the insect pathogens and *B. anthracis* may be more remote than for *B. cereus* is suggested both by the notably fewer number of anthrax strains yielding any agglutination at all and by the lower order of titers obtained with positively reacting strains.

Agglutination tests with the insect pathogens using antisera against spores of strains of *B. cereus* and *B. anthracis* were performed to seek confirmation of the existence of spore agglutinogens held in common (Table 2). The data make it obvious that the specificity of antisera induced by injection of spores is not homologous with the species from which the spores are obtained for injection of rabbits. Clearly the spore agglutination test provides no sharp lines of demarcation for separating the three kinds of organisms.

To determine whether some systematic, readily discernible pattern of distribution of agglutinogens exists among the species, a series of cross adsorption trials was undertaken. The results obtained when spores of two strains of *B. anthracis* were employed to adsorb anti-

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TABLE 1

Agglutinin titers of antisera prepared against the endospores of insect pathogenic bacilli employing endospores from the indicated species as agglutinogens

Spore Agglutinin Species and Strain	Insect Pathogenic Species							*Bacillus cereus*									*Bacillus anthracis*			
	Bacillus thuringiensis					*Bacillus entomocidus*														
	var. *thuringiensis*		var. *alesti*		var. *sotto*	var. *entomocidus*	var. *subtoxicus*													
	0-3-30	16-2-2	0-24-3	0-32-2	0-28-1	57-1-1	58-1-1	Ba2(22)	Ba2(16)	NRS201	NRS1256	NRS617	NRS232	NRS244	NRS793	NRS249	Carbozoo	380	385	391
B. thuringiensis																				
var. *thuringiensis*																				
0-3-30	**2048**	4096	128	256	128	0	64	64	512	128	32	1024	256	0	4096	4096	64	0	0	16
16-2-2	4096	**4096**	64	128	512	64	64	64	128	128	32	1024	256	0	4096	4096	32	SA*	0	4
var. *alesti*																				
0-24-3	64	128	**1024**	64	512	0	128	0	256	0	32	0	2048	128	2048	128	0	256	0	0
0-32-2	0	0	256	**2048**	512	64	256	0	0	1024	8	64	128	0	128	16	128	64	0	0
var. *sotto*																				
0-28-1	8	0	256	4096	**1024**	0	512	0	1024	2048	128	32	128	256	2048	32	32	0	32	0
B. entomocidus																				
var. *entomocidus*																				
57-1-1	0	32	128	256	64	**1024**	32	4	1024	512	1024	32	2048	1024	256	8	32	16	4	SA*
var. *subtoxicus*																				
58-1-1	16	16	128	512	256	8	**256**	0	0	32	0	16	128	16	256	8	8	0	16	4

Titers for the homologous spore agglutinogen are recorded in bold face. In addition to the strains of *B. anthracis* listed, 17 other strains were tested. With 6 of these, agglutination was not obtained; 4 gave only a low titer agglutination with 1 antiserum; 7 showed either no agglutination or spontaneous agglutination with the different antisera.

* Spontaneous agglutination.

TABLE 2

Agglutinin titers with endospores of insect pathogens employing spore antisera prepared against strains of Bacillus cereus and Bacillus anthracis and adsorbed with homologous vegetative bacilli

Spore Antisera	Homologous Titer	Heterologous Spore Agglutinogen						
		Bacillus thuringiensis					*Bacillus entomocidus*	
		0-3-30	16-2-2	0-24-3	0-32-2	0-28-1	57-1-1	58-1-1
B. cereus								
NRS201	>1024	0	256	512	128	>1024	0	>1024
NRS946	>1024	0	0	0	128	0	0	0
NRS232	>1024	64	128	512	>1024	>1024	>1024	>1024
NRS244	1024	128	64	256	>1024	512	>1024	>1024
NRS617	1024	>1024	>1024	64	256	64	32	32
Ba2(16)	>1024	8	8	256	>1024	128	1024	512
NRS1256	>1024	128	256	64	256	32	256	16
Ba2(19)	>1024	>1024	0	4	0	0	0	0
NRS793	>1024	512	512	512	>1024	>1024	64	512
Ba2(23)	>1024	0	0	>1024	>1024	>1024	512	>1024
NRS847	SA	>1024	>1024	0	64	0	16	0
Ba2(22)	256	0	0	0	32	0	0	0
B. anthracis								
Carbozoo	2048	8	64	8	64	64	0	8
400	1280	128	256	32	256	128	32	128
390	1024	0	16	0	32	16	0	8

TABLE 3

Agglutinin titers of antisera against spores of insect pathogens adsorbed with spores of Bacillus anthracis

Spore Agglutinogen	Antiserum Adsorbed								
	Bacillus entomocidus 57-1-1			*Bacillus thuringiensis*					
				0-3-30			0-24-3		
	Before adsorption	Adsorption with Carbozoo	Adsorption with 380	Before adsorption	Adsorption with Carbozoo	Adsorption with 380	Before adsorption	Adsorption with Carbozoo	Adsorption with 380
Insect pathogen									
0-3-30				1024	0	0	16	0	0
16-2-2	0	0	0	1024	0	16	16	0	32
0-32-2	128	0	0	32	0	0	512	0	256
0-28-1	64	0	0	16	0	0	32	0	64
0-24-3	64	0	0	16	0	0	512	0	128
57-1-1	16	0	0	0	0	0	0	0	0
58-1-1	0	0	0	32	0	0	64	0	32
B. anthracis									
Carbozoo	32	0	0	32	0	0			
380	256	0	0				512	0	0
Bacillus cereus									
NRS232	512	0	64	32	8	16	1024	64	64
NRS244	256	0	256				256	0	0
NRS249				512	32	128	64	0	256
NRS793	256	16	16	256	8	32	1024	16	128
NRS201	256	32	16	16	0	0			
NRS1256	512	64	0	0	0	0	64	0	32
NRS617	256	0	0	128	0	0			
Ba2(16)	256	128	512	64	0	0	512	64	64

TABLE 4

Adsorption of antisera against spores of insect pathogens and Bacillus cereus with spores of heterologous strains

Data recorded are the number of strains for which the agglutinin titer was reduced to zero to the total number of strains tested exclusive of the strain used for adsorption.

Adsorbing Spore Antigen	Serum Adsorbed									
	Bacillus thuringiensis				*Bacillus entomocidus* 57-1-1		*B. cereus*			
	0-3-30		0-24-3				NRS232		NRS793	
	Species for spore agglutination									
	Insect pathogens	*B. cereus*	Insect pathogens	*B. cereus*	Insect pathogens	*B. cereus*	Insect pathogens	*B. cereus*	Insect pathogens	*B. cereus*
0-3-30			0/4	2/6	3/4	3/7	0/4	1/10	1/5	3/6
0-24-3	3/5	3/8			3/3	2/6	2/3	2/10	3/5	3/6
57-1-1	0/6	3/8	0/4	3/6			1/4	4/10	1/6	2/6
NRS232	1/6	5/6	3/6	4/5	4/4	6/6				

body from antisera prepared against three strains of insect pathogens are recorded in Table 3. Data are also included on how the adsorption affected titers against spores of eight strains of *B. cereus*. No simple relationships can be derived from these data which show that results with one strain as adsorbent are not necessarily duplicated by the use of another strain of *B. anthracis*. Table 4 attempts to summarize results recorded when antisera against spores of insect pathogens and *B. cereus* were adsorbed with spores from the heterologous species. These adsorption experiments demonstrate the antigenic complexity of the spores and the existence of a complicated pattern of distribution of antigens among the three kinds of organisms. Based on past experience (Lamanna and Eisler, 1960), it might be expected that if a larger battery of strains were subjected to analysis, the existence of interspecies and intraspecies relationships woud appear even more complex.

In spite of the antigenic cross reactions demonstrated in this paper and the recent report of Yoder and Nelson (1960) on the existence of bacteriophage which can cross the host range boundary between *B. thuringiensis* Berliner, and *B. anthracis*, we believe it would be premature for taxonomic purposes to consider the organisms under discussion as a single species. Particularly in the case of *B. anthracis*, there are a number of characteristics aside from pathogenicity which justify its continued treatment as a distinct and recognizable species (Burdon, 1956; Leise et al., 1959). In this connection it is of interest to know that neither by laboratory exposure nor in field trials as a live pest control agent against susceptible insect species has *B. thuringiensis* Berliner shown any signs of pathogenicity for man and other animals susceptible to anthrax (Fisher and Rosner, 1959).

SUMMARY

Agglutination and agglutinin adsorption tests have revealed the existence of antigenic relationships among the endospores of the crystalliferous insect pathogenic spore-bearing bacilli and *Bacillus cereus* and *Bacillus anthracis*. It has not proved possible to differentiate among these organisms by means of serological agglutination tests employing endospores as agglutinogens.

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