SIMILARITY IN BASE COMPOSITIONS OF DEOXYRIBONUCLEATES FROM SEVERAL STRAINS OF *BACILLUS CEREUS* AND *BACILLUS ANTHRACIS*

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ABSTRACT

McDONALD, WILLIAM C. (U.S. Army Biological Laboratories, Frederick, Md.), IRA C. FELKNER, ABRAHAM TURETSKY, AND THOMAS S. MATNEY. Similarity in base compositions of deoxyribonucleates from several strains of *Bacillus cereus* and *Bacillus anthracis*. J. Bacteriol. **85**:1071–1073. 1963.—Studies were made on the base compositions of the deoxyribonucleates of a number of *Bacillus* species. Species homogeneity was quite evident from these studies since, with only one exception, several strains of the same species showed identical base compositions. It was found that the melting temperatures and corresponding percentages of guanine plus cytosine were quite similar in several strains of *B. cereus* and *B. anthracis*. These aspects are discussed as related to their possible genetic homology.

The relationship between genetic homology and deoxyribonucleic acid (DNA) base compositions has been discussed by Falkow, Ryman, and Washington (1962) and Schildkraut, Marmur, and Doty (1961). In the present study, we examined the compositions of some members of the genus *Bacillus*. It was desired to determine whether any other species of the genus *Bacillus* had base composition similar enough to that of *B. anthracis* to suggest genetic homology. The results are discussed, relating the base compositions to phenotypic characteristics which are employed in classification.

MATERIALS AND METHODS

Cultures. The following organisms were used: *B. cereus* Lamanna, *B. cereus* 7064, *B. cereus* W, *B. cereus* 9139, *B. cereus* 68, *B. cereus* 569, *B. anthracis* HBA87, *B. anthracis* 30R/S^r, *B. licheniformis* Allen, *B. licheniformis* CDII, *B. licheniformis* 9945A, *B. subtilis* 23, *B. subtilis* 23-1, *B. subtilis* P1, *B. subtilis* T7, *B. subtilis* 168, and *B. subtilis* 168/S^r. All strains mentioned came from stock culture collections maintained at this installation.

Isolation of DNA. DNA was isolated in the following manner from lysozyme-insensitive strains which included all *B. anthracis* and *B. cereus* strains studied, except *B. cereus* 569. Packed vegetative cells (1 to 3 g) were washed three times with 25 ml of 0.15 M NaCl plus 0.1 M ethylenediaminetetraacetic acid (EDTA; pH 8), and the supernatant liquid was poured off after centrifugation at 4 C. The cells were then suspended in 25 ml of 0.15 M trichloroacetate (pH 8, prepared by adding NaOH to trichloroacetic acid) and shaken for 30 to 40 min at room temperature. (After such treatment, the cells appeared to lose their gram-positive staining characteristic.) A 2-ml amount of 25% sodium lauryl sulfate was added, and the suspension was heated at 60 C for 10 min. Lysis was indicated by a marked increase in viscosity. An equal volume of phenol was then added, and the emulsion was shaken for 1 min at room temperature. If good lysis was not evident, the emulsion was shaken for 30 to 40 min. The emulsion was then centrifuged in conical centrifuge tubes until three layers formed (after approximately 10 min). The top layer, which contained the crude nucleic acids, was withdrawn and overlayed with twice its volume of 95% ethanol. The precipitating strands were wound on a glass rod and dissolved in standard saline-citrate (Marmur, 1961). The material was re-extracted with an equal volume of 90% phenol, centrifuged, and precipitated with ethanol until the interface was eliminated. [The procedure proposed by Sevag, Lackman, and Smolens (1938) may also be used for re-extrac-

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TABLE 1. *Comparison of base compositions of Bacillus cereus, B. anthracis, and some other Bacillus species*

Strain	Tm	Deviation from mean Tm	Per cent guanine plus cytosine	Deviation from mean % G-C
	C			
B. cereus Lamanna	82.00	0.25	31.70	0.50
B. cereus 7064	82.25	0.00	32.10	0.10
B. cereus W	82.25	0.00	32.10	0.10
B. cereus 9139	82.30	0.05	32.40	0.20
B. cereus 68	82.45	0.20	32.80	0.60
Mean *B. cereus* value	82.25	±0.11	32.20	0.30
B. cereus 569*	85.45	—	40.1	—
B. anthracis HBA87	82.60	0.14	32.20	0.35
B. anthracis 30R/S^r	82.88	0.14	33.90	0.35
Mean *B. anthracis* value	82.74	±0.14	33.55	0.35
B. licheniformis Allen	86.60	0.16	42.90	0.67
B. licheniformis CDII	86.62	0.14	43.00	0.23
B. licheniformis 9945A	86.95	0.19	43.80	0.57
Mean *B. licheniformis* value	86.76	±0.16	43.23	0.49
B. subtilis 23	87.75	0.15	45.70	0.60
B. subtilis 23-1	87.75	0.15	45.70	0.60
B. subtilis P1	87.80	0.10	45.90	0.80
B. subtilis T7	87.90	0.00	46.10	0.00
B. subtilis 168	88.10	0.20	46.60	0.50
B. subtilis 168/S^r	88.10	0.20	46.60	0.50
Mean *B. subtilis* value	87.90	±0.14	46.10	0.50

* *B. cereus* 569 did not fit into the pattern of the other *B. cereus* strains analyzed and therefore was not considered in the mean value.

tion.] From this point on, the procedure followed the final steps outlined by Marmur (1961) to obtain highly purified DNA. All other species studied, which included *B. cereus* 569, were sensitive to lysozyme and were isolated by the method of Marmur (1961). All DNA samples were dissolved in standard saline-citrate (SSC; 0.15 M NaCl + 0.015 M Na citrate; pH 7.0) and stored at 4 C over a few drops of chloroform.

Determination of base composition of DNA of various species. The procedure used for the determination of the melting temperature (Tm) was as outlined by Falkow et al. (1962). The formula, % G − C = (Tm-69) 2.439, can be used for calculating the per cent of guanine plus cytosine if one has the Tm of a given species or strain. In this formula, the 69 represents the melting point of a pure adenine plus thymine polymer (Marmur and Doty, 1959), and every degree rise in the Tm above 69 C represents an increase of 2.439% in the G − C content.

RESULTS AND DISCUSSION

The results indicate that species of the genus *Bacillus* fall into distinct groups when they are analyzed with respect to the base composition of their DNA. The data further show a similarity to *B. anthracis* in the base composition of the deoxyribonucleates from all but one of the *B. cereus* strains (strain 569) tested. Strains of *B. subtilis* and *B. licheniformis* displayed good homogeneity within their own groups but differed distinctly from the other species.

The Tm values for individual *Bacillus* species are shown in Table 1, and the mean value for each species is indicated. The corresponding guanine plus cytosine (G − C) values are also indicated.

Excluding strain 569, the mean Tm obtained for *B. cereus* strains was 82.25 C with the average deviation of each strain being ±0.11 C. The corresponding % G − C was 32.2% with average strain differences being ±0.30%. It is interesting to note that *B. cereus* 569 had a Tm of 85.45 C, which corresponds to 40.1% G − C. For this reason, it was not included with the rest of the *B. cereus* strains analyzed.

The average Tm for *B. anthracis* strains tested was 82.74 C, with each strain varying ±0.14 C from this mean. The mean % G − C was 33.5% with variation being ±0.35% in each of the two strains analyzed.

The mean Tm for *B. licheniformis* strains was determined to be 86.7 C with an average deviation of ±0.16 C for individual strains. The corresponding % G − C was 43.23% with average strain deviation being ±0.49%.

In the *B. subtilis* strains tested, the mean Tm was 87.90 C with an average variation of ±0.14 C among strains. The mean % G − C was 46.10% and average deviation was ±0.50%.

It is of interest to note that strains of *B. cereus* and *B. anthracis* which are on extreme ends, i.e., *B. cereus* Lamanna and *B. anthracis* 30R/S^r, are different by 0.88 C in their Tm and differ in their % G − C by 2.20%. However, *B. anthracis* HBA87 differs from *B. cereus* 68 by only 0.15 C with the difference in % G − C being 0.40%

The mean difference in Tm between *B. anthracis* and *B. cereus* is 0.49 C, and the corresponding difference in % G − C is 1.35%.

The similarity in the base compositions of deoxyribonucleates from most of the strains of *B. cereus* analyzed to those of *B. anthracis* strains indicates that these species are closely related. Falkow et al. (1962) have stated the following. "The proposition that genetic homology is related to equality of DNA base composition does not imply that all organisms with similar base compositions will exhibit genetic compatibility. Similarity of composition is, however, a minimal requirement." In the case of *B. cereus* and *B. anthracis*, this similarity may be more meaningful in that many phenotypic characteristics of these species are quite close. This close phenotypic relationship was suggested by Clark (1937) and was emphasized by Breed, Murray, and Smith (1957). There is, however, one notable exception to the DNA base pattern in the *B. cereus* group. This is *B. cereus* strain 569, which possibly should not be classified as *B. cereus*.

In conclusion, two observations are apparent. First, there is remarkable homogeneity of base compositions within groups of *Bacillus* species. Second, *B. cereus* and *B. anthracis* strains form a homogeneous group but differ markedly from the other *Bacillus* species tested. The distinction made between *B. cereus* and *B. anthracis* to date is based largely on pathogenicity, although phage sensitivity is sometimes used (Brown and Cherry, 1955). Although the former criterion may be valid for strain differentiation, it should not be used to define a species which connotes those organisms which are genetically compatible. Although phage sensitivity is reliable, there are instances in which phages will not attack some members of a given species because of resistance or by virtue of lysogeny. Since the gross base compositions of the *B. cereus* and *B. anthracis* strains tested are quite similar, we may be able to predict that some strains of these organisms which have been previously classified as different species could be genetically compatible.

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