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Fatty Acids in the Genus *Bacillus*

II. Similarity in the Fatty Acid Compositions of *Bacillus thuringiensis*, *Bacillus anthracis*, and *Bacillus cereus*[1]

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The nature and relative abundance of fatty acids produced by two strains each of *Bacillus thuringiensis* and of *B. anthracis* were studied by gas-liquid chromatography on a 12,000 theoretical plate polyester column capable of partially resolving iso- and anteiso-fatty acids with the same number of carbon atoms. Unsaturated fatty acids as the bromo derivatives were separated from the saturated acids and resolved in a short SE-30 column by use of programmed-temperature gas chromatography. All four strains produced 16 major fatty acids: 9 branched (i-C_{12}, i-C_{13}, i-C_{14}, i-C_{15}, i-C_{16}, i-C_{17}, a-C_{13}, a-C_{15}, and a-C_{17}), 3 normal (n-C_{14}, n-C_{15}, and n-C_{16}), and 4 monounsaturated (i-$C_{16}^{1=}$, i-$C_{17}^{1=}$, a-$C_{17}^{1=}$, and n-$C_{16}^{1=}$), in addition to some minor fatty acids. In all cases, 12 branched acids, including saturated and monounsaturated, made up over 70% of the total fatty acids, and iso-C_{15} acid was most abundant. These fatty acid distribution patterns were very similar to those of *B. cereus* and *B. cereus* var. *mycoides*. There were, however, minor but clear differences between the fatty acid distribution patterns of *B. thuringiensis* and *B. anthracis*. *B. thuringiensis*, like *B. cereus*, produced higher proportions of i-C_{13}, a-C_{13}, and i-C_{14} fatty acids than did *B. anthracis*. This difference between these two species could be useful as a supplemental criterion in their differentiation. Indications are that the enzyme systems for monounsaturated fatty acid synthesis in *B. thuringiensis* and *B. anthracis* prefer normal fatty acids as substrates rather than branched-chain fatty acids.

Previous work from this laboratory (6) has shown that all of 22 strains of 10 species of the genus *Bacillus* examined produced branched-chain fatty acids, 14- to 17-carbon iso and anteiso series, as the major components of the total fatty acids. *B. cereus* alone, however, was quite distinctive, producing four additional fatty acids, including one major unsaturated fatty acid. [Iso (i) and anteiso (a) refer to a methyl side chain in the penultimate and antepenultimate positions, respectively, as compared with the normal (n) fatty acids.]

B. thuringiensis and *B. anthracis* are taxonomically very close to *B. cereus*. Smith et al. (9) even considered them as pathogenic varieties of *B. cereus* or vice versa, although subsequently Burdon (1) and Leise et al. (7) reported some taxonomic distinctions. Therefore, it is of interest to examine these less common but pathogenically well-known bacilli to determine whether they also produce terminally methyl branched fatty acids as the major component of the total fatty acids. Their fatty acid compositions can then be compared with that of *B. cereus* to see whether similarity in taxonomy is reflected in biochemical activity.

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MATERIALS AND METHODS

Microorganisms. Sources and original designations of the cultures used are listed in Table 1. Homogeneity of all the cultures upon reception was examined by plating on blood-agar medium. With the exception of *B. anthracis* 5.4, all cultures were homogeneous. This culture, which was originally isolated from a cow victim in Quebec, showed two clearly distinguishable types of colonies, one of which was confirmed as *B. anthracis* by various standard tests (1), including negative hemolytic activity. The *B. anthracis* colonies were separated and used in the experiments. All organisms were maintained as stock cultures on Nutrient Agar (Difco) slants.

Culture media. Culture media used to grow the organisms for the isolation of fatty acids were a glucose (1%)-yeast extract (0.1%, Difco) medium (6) and Pennassay Broth (Difco). To prepare slants, 2% agar (Difco) was added to the glucose-yeast extract medium.

Isolation of fatty acids from the organisms. B.

TABLE 1. *Cultures of Bacillus thuringiensis and B. anthracis*

Present designation	Name	Original designation	Source
B. thuringiensis (I)	*B. thuringiensis* var. *thuringiensis* Berliner	—	W. Simirnoff[a]
B. thuringiensis (II)	*B. thuringiensis*	B-2172	W. C. Haynes[b]
B. anthracis (I)	*B. anthracis*	Anthracis 5.4	R. Filion[c]
B. anthracis (II)	*B. anthracis*	Anthracis PHL	J. I. Payne[d]

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[b] Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Ill.

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thuringiensis was grown on glucose-yeast extract broth and agar and on Pennassay Broth, whereas *B. anthracis* was grown only on glucose-yeast extract-agar. With the liquid media (the glucose-yeast extract and the Pennassay Broth), procedures identical to those described earlier (6) were used to grow the organisms and to isolate fatty acids. With the glucose-yeast extract-agar medium, the following procedures were used. The organisms were grown on slants of 80 ml of the agar medium in 250-ml Erlenmeyer flasks at 37 C for 2 days. A 25-ml amount of 0.85% NaCl solution was added to each flask, and the cell suspensions thus obtained were transferred to 250-ml round-bottom flasks. The suspensions in the flasks were steamed for 15 min at 15 psi to kill all the viable cells, and after the addition of 50 ml of methanol and 0.5 g of KOH, were refluxed for 4 hr. The saponified cells were treated with *n*-hexane to extract neutral and basic materials, and then were acidified and treated with *n*-hexane to extract fatty acids. Fatty acids thus isolated were methylated with diazomethane and analyzed by gas-liquid chromatography as before (4).

Gas-liquid chromatography. Two systems were used to identify and estimate bacterial fatty acids. A 20-ft (6.1-meter) ethylene glycol adipate polyester column [7% of the polyester on Anakrom ABS 60/80 mesh (Analabs, Inc., Hamden, Conn.) packed in ⅛-inch (0.3-cm) outer diameter copper tubing] was used with a Hewlett-Packard model 700 chromatograph. The column temperature was 170 C, and the helium flow rate was 2 to 4 ml/min.

A 5-ft (1.5-meter) silicon gum rubber column [2.5% of SE-30 on Chromosorb G-A.W.-DMCS 80/100 mesh (Perkin-Elmer) packed in ⅛-inch outer diameter stainless steel tubing] was used with a MicroTech model 2000 R-GC chromatograph. The helium flow rate here was 20 ml/min, and the temperature was kept for 2 min at 130 C and then programmed at a rate of 5 C per min up to 260 C. Flame ionization detectors were used in both cases.

Growth. Growth of the organisms on liquid media was measured by use of a Klett-Summerson colorimeter with a no. 66 filter. The growth on the agar slants was not determined, but in all cases the cells completely covered the slant surface with a thick layer.

Standard fatty acids. Standards of branched-chain fatty acids and of normal fatty acids prepared during earlier work (4) were also used in the present work, in addition to normal fatty acids purchased from Applied Science Laboratories, Inc., State College, Pa., and Sigma Chemical Co., St. Louis, Mo. Fatty acids of *B. subtilis* (ATCC 7059) and of *B. cereus* (B 108) which have been identified previously by various physical methods, including mass spectrometry, infrared spectroscopy, and gas-liquid chromatography, were also used as positional standards. The methyl ester of tridecanoic acid, which was not produced by either species to any measurable extent, was added to the washed cells before saponification and was used as the internal standard to determine the amounts of fatty acids present in the original cell sample.

RESULTS

Identification of bacterial fatty acids. Thirteen peaks, designated 1 to 13 in Fig. 1, were assigned to saturated fatty acids because (i) the peaks were not diminished at all by catalytic hydrogenation or bromination (3) prior to the injection, and (ii) the apparent chain lengths of these bacterial fatty acids determined against standard normal fatty acids (8) on adipate columns were almost identical to those measured on an SE-30 column, indicating the absence of polar functional groups and unsaturated carbon linkages. Cochromatography of these fatty acids with the standard normal and branched fatty acids on the adipate and SE-30 columns has led to the conclusion that they are i-C_{12}, n-C_{12}, i-C_{13}, a-C_{13}, i-C_{14}, n-C_{14}, i-C_{15}, a-C_{15}, n-C_{15}, i-C_{16}, n-C_{16}, i-C_{17}, and a-C_{17} acids (Table 2).

Peaks designated a, b, c, and d in Fig. 1 were assigned to monounsaturated fatty acids by the following observations: the apparent chain length of each of these four fatty acids on the adipate column was 0.4 to 0.5 carbon units larger than that measured on the SE-30 column. When the fatty acids were reduced, the apparent chain length of each reduced fatty acid was almost identical on both columns, and this value was nearly identical to the average of the apparent chain length of the original fatty acid on the two columns.

Cochromatography of the reduced samples of these fatty acids against the standard fatty acid samples indicates that the reduced fatty acids

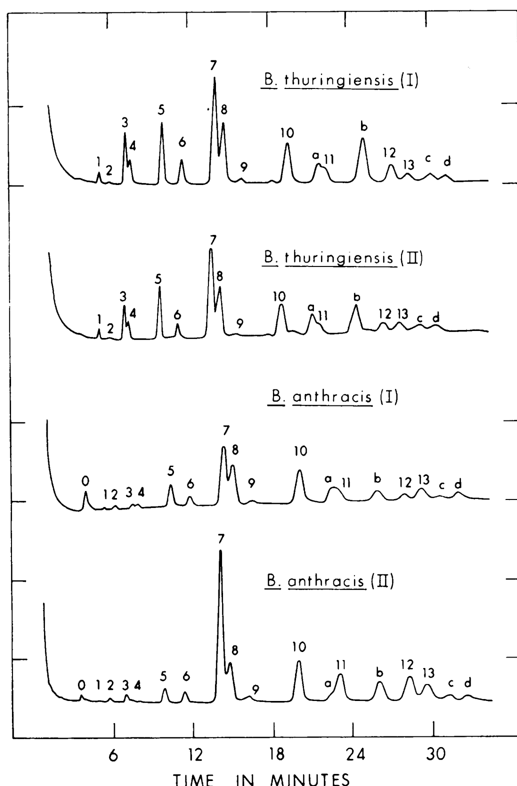

FIG. 1. *Gas-liquid chromatograms of bacterial fatty acids obtained under isothermal conditions (170 C) on an adipate column (20 ft). For the top two chromatograms, the fatty acids samples corresponded to 8 ml of the submerged culture (100 ml total) of Bacillus thuringiensis, whereas for the bottom two the fatty acid samples corresponded to 4% of the agar cultures of B. anthracis.*

are, respectively, i-C_{16}, n-C_{16}, i-C_{17}, and a-C_{17} acids. Consequently, peaks a, b, c, and d correspond to monounsaturated homologues of i-C_{16}, n-C_{16}, i-C_{17}, and a-C_{17} acids (Table 3). The identification of these acids is further confirmed by comparing the original unsaturated fatty acid samples with the corresponding unsaturated fatty acids of *B. cereus* (6). The fatty acids corresponding to peaks a, b, c, and d were further identified by means of their bromo derivatives. When the total fatty acid mixture was treated with Br_2 in cold ethyl ether (3) and then chromatographed, these four peaks disappeared from the original positions in the chromatograms on the adipate column, but under programmed-temperature gas-liquid chromatography on the SE-30 column four new peaks corresponding to the brominated fatty acids were observed at much higher retention temperatures. Figures 2A and 2B are chromatograms obtained with the total fatty acid mixtures of *B. thuringiensis* (I) and *B. anthracis* (I) before and after brominations. Cochromatography of the brominated fatty acids with a standard, 9,10-dibromomethylpalmitate (prepared from methyl-palmitoleate), gave further evidence that the original acids were monounsaturated derivatives of i-C_{16}, n-C_{16}, and i-C_{17} plus a-C_{17} acids, and the new peaks were their dibromo derivatives.

This method is an efficient and simple way to determine unsaturated fatty acids present in a

TABLE 2. *Gas-liquid chromatographic apparent chain length of saturated fatty acids isolated from Bacillus thuringiensis and B. anthracis*

Peak[a]	Fatty acid[b]	Apparent chain length of bacterial fatty acid as methyl ester	
		On adipate column	On SE-30 column
1	i-C_{12}	11.62	11.6
2	n-C_{12}	12.00	12.0
3	i-C_{13}	12.58	12.6
4	a-C_{13}	12.72	12.7
5	i-C_{14}	13.57	13.6
6	n-C_{14}	14.00	14.0
7	i-C_{15}	14.60	14.6
8	a-C_{15}	14.74	14.7
9	n-C_{15}	15.00	15.0
10	i-C_{16}	15.57	15.6
11	n-C_{16}	16.00	16.0
12	i-C_{17}	16.58	16.6
13	a-C_{17}	16.73	16.7

[a] Peak numbers as indicated on the chromatograms (Fig. 1).
[b] Fatty acids are designated by the total number of carbon atoms as normal, iso, and anteiso.

TABLE 3. *Gas-liquid chromatographic apparent chain length of unsaturated fatty acids isolated from Bacillus thuringiensis and B. anthracis*

Peak[a]	Fatty acid[a]	Apparent chain length of bacterial fatty acid as methyl ester			
		On adipate column		On SE-30 column	
		Original	Reduced	Original	Reduced
a	i-$C_{16}^{1=}$[b]	15.90	15.57	15.4	15.6
b	n-$C_{16}^{1=}$	16.32	16.00	15.8	16.0
c	i-$C_{17}^{1=}$	16.88	16.58	16.4	16.6
d	a-$C_{17}^{1=}$	16.97	16.73	16.5	16.7

[a] See Table 2.
[b] i-$C_{16}^{1=}$ represents 14-methylpentadecanoic acid with one unsaturated carbon bond at a position which has not been determined.

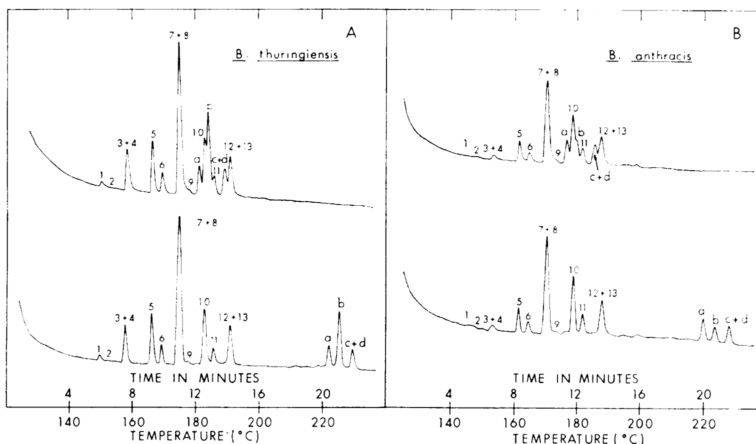

FIG. 2. *Gas-liquid chromatograms of Bacillus thuringiensis fatty acids (A) and of B. anthracis fatty acids (B) obtained under programmed-temperature operation (130 to 260 C at a rate of 5 C per min) on an SE-30 column (5 ft in length). (A) Eight per cent of the total fatty acids isolated from 100 ml of the submerged culture of B. thuringiensis (I) was used before bromination for the top chromatogram and after bromination for the bottom one. (B) Four per cent of the total fatty acids isolated from the agar culture of B. anthracis (I) was used before bromination for the top chromatogram, and after bromination for the bottom one.*

given fatty acid sample and may supplement the standard method that involves comparison of apparent chain lengths measured on polar and nonpolar columns. This is also a better way to determine accurately amounts of saturated acids by eliminating interference from imperfectly resolved peaks of unsaturated fatty acids. Gas-liquid chromatographic peak areas of the bromo derivatives, as observed with the flame ionization detector, appear to be smaller than the corresponding peak areas of the original fatty acids.

Fatty acid distribution patterns of B. anthracis and of B. thuringiensis grown on glucose-yeast extract slants. Kaneda (5) has shown that the fatty acid distribution pattern of *B. subtilis* (ATCC 7059) changes greatly, depending upon the age of the cells harvested. For this reason, it would be preferable to let a test organism grow on a liquid medium rather than a solid medium. Because of pathogenicity of the organism, *B. anthracis* was grown only on a solid medium to minimize handlings, and *B. thuringiensis* (II) grown under identical conditions was used to make a direct comparison between the two species.

Table 4 shows fatty acid distribution patterns of two strains of *B. anthracis* (I and II) and of *B. thuringiensis* (II) grown on glucose-yeast extract slants. They are all very similar in pattern, showing 17 major peaks including 12 saturated and 4 monounsaturated fatty acids. In addition, both strains of *B. anthracis* produced a small proportion of additional fatty acids (Fig. 1), peak 0, presumably i-C_{11} and a-C_{11} acids. In all cases, the sum of branched-chain fatty acids, including saturated and unsaturated, is 70 to 80% of the total fatty acids, and i-C_{15} acid is more than a-C_{15} acid. There are, however, minor but clear differences between these two species. The acids i-C_{12}, i- and a-C_{13}, and i-C_{14} were produced in higher proportions in *B. thuringiensis* than in *B. anthracis*, whereas the a-C_{17} acid and monounsaturated a-C_{17} acid were produced in lower proportions in the former than in the latter. Some differences were also observed between two strains of *B. anthracis* with such acids as i-C_{14}, i- and a-C_{15}, monounsaturated i-C_{16}, and i-C_{17} (Table 4). This is indicative of differences in metabolic activities related to the synthesis of these branched-chain fatty acids between these two strains, presumably in the metabolism of branched-chain amino acids (5). In contrast, the two strains of *B. thuringiensis* gave almost identical proportions of fatty acids when they were grown under the same culture conditions.

Fatty acid distribution patterns of B. thuringiensis grown on glucose-yeast extract or Pennassay Broth media. Table 5 shows the relative proportions of the combined odd-numbered iso, the combined even-numbered iso, the combined anteiso (odd-numbered), and the combined normal fatty acids. The theoretical reason why the bacterial fatty acids are grouped into these four classes is that the fatty acids in the same class are very closely related to each other in their biosynthetic pathway. Detailed discussion of this theory appeared in an earlier paper (6).

TABLE 4. *Fatty acid distribution patterns of Bacillus thuringiensis and B. anthracis grown on glucose-yeast extract slant (GYA) or broth (GY)*

Organism[a]	Culture medium	Percentage of the total fatty acids[b]																	
		$i\text{-}C_{12}$	$n\text{-}C_{12}$	$i\text{-}C_{13}$	$a\text{-}C_{13}$	$i\text{-}C_{14}$	$n\text{-}C_{14}$	$i\text{-}C_{15}$	$a\text{-}C_{15}$	$n\text{-}C_{15}$	$i\text{-}C_{16}$	$i\text{-}C_{16}^{1=}$	$n\text{-}C_{16}$	$n\text{-}C_{16}^{1=}$	$i\text{-}C_{17}$	$a\text{-}C_{17}$	$i\text{-}C_{17}^{1=}$	$a\text{-}C_{17}^{1=}$
B. anthracis (I)	GYA	0.2	0.5	1.0	0.7	5.9	2.7	19.4	15.6	0.9	13.5	6.2	6.2	5.9	3.5	5.7	2.3	4.6
B. anthracis (II)	GYA	0.0	0.5	0.9	0.0	2.5	1.8	31.4	9.8	0.9	11.6	2.4	9.1	6.8	10.0	7.6	2.4	2.2
B. thuringiensis (II)	GYA	1.1	0.2	4.5	1.7	11.7	2.2	15.2	7.1	0.8	15.8	7.0	7.0	8.2	6.4	2.8	1.9	0.9
	GY	1.2	0.0	8.0	3.2	9.2	3.4	20.7	11.0	0.4	8.1	5.4	1.7	13.2	5.5	1.9	2.7	1.3

[a] The organisms were grown either for 48 hr at 37 C on 80 ml of glucose-yeast extract slant (see the text for detail) or for 16 hr at 37 C in 100 ml of glucose-yeast extract broth on a shaker. The cells were washed once with a 0.85% NaCl solution and analyzed for fatty acids by gas-liquid chromatography as described in text.

[b] See Table 2 for the abbreviations used for fatty acids.

TABLE 5. *Relative abundances of four fatty acid classes of Bacillus thuringiensis grown on glucose-yeast extract medium (GY) or on Pennassay Broth (PB)*

Organism	Medium	Percentage of the total fatty acids[a]				Growth[b] (Klett units)	Total fatty acids in 100 ml of culture (mg)
		$i\text{-}C_{12} + i\text{-}C_{14} + i\text{-}C_{16} + i\text{-}C_{16}^{1=}$	$i\text{-}C_{13} + i\text{-}C_{15} + i\text{-}C_{17} + i\text{-}C_{17}^{1=}$	$a\text{-}C_{13} + a\text{-}C_{15} + a\text{-}C_{17} + a\text{-}C_{17}^{1=}$	$n\text{-}C_{12} + n\text{-}C_{14} + n\text{-}C_{16} + n\text{-}C_{16}^{1=}$		
B. thuringiensis (I)	GY	23.9	36.9	17.5	18.3	206, 206	3.1, 3.6
	PB	16.6	46.8	14.5	19.6	170, 169	1.7, 2.4
B. thuringiensis (II)	GY	28.9	35.4	21.4	15.2	292, 294	2.8, 2.9
	PB	12.0	53.9	14.3	17.4	320, 317	4.9, 5.6

[a] See Table 2 for the abbreviations used for fatty acids.

[b] The organisms were grown for 16 hr at 37 C in 100 ml of either medium on a shaker. One Klett unit corresponds to 0.50 mg (dry weight) of cells per 100 ml of culture.

B. thuringiersis, when grown on glucose-yeast extract medium, produced four classes of fatty acids, the combined odd-numbered iso (related to leucine), the combined anteiso (related to isoleucine), the combined even-numbered iso (related to valine), and the combined normal, in decreasing order of relative abundance. The last two classes were present in a smaller and similar abundance, and their relative abundances between the two strains were found to be reversed. When Pennassay Broth, an amino acid-rich medium, was used to grow *B. thuringiensis*, in both cases the relative proportion of the combined odd-numbered iso acids increased greatly, to nearly 50% of the total fatty acids, and that of the combined normal fatty acids also increased slightly; in contrast, the proportions of the combined anteiso fatty acids and of the combined even-numbered iso fatty acids decreased significantly. A similar effect with Pennassay Broth has been observed with the other 10 bacilli (6).

Relative abundance of unsaturated fatty acids in relation to their saturated homologues. Unsaturated fatty acids occur in *B. thuringiensis* and *B. anthracis* to the extent of 14 to 20% of the total fatty acids. Table 6 shows relative proportions of the four major unsaturated fatty acids and of the corresponding four saturated fatty acids. Among the unsaturated fatty acids, $n\text{-}C_{16}$ occurs most abundantly, whereas among the saturated fatty acids $i\text{-}C_{16}$ or $i\text{-}C_{17}$ is most abundant. The ratios of each unsaturated fatty acid to the corresponding saturated fatty acid are also listed. The ratio for the $n\text{-}C_{16}$ homologues was much higher and also more dependent on the growth condition than the ratios for the remaining three pairs of fatty acids. As will be discussed later, this means that, if each unsaturated fatty acid is produced by desaturation of the corresponding saturated fatty acid, $n\text{-}C_{16}$ acid is the most active of the four C_{16} and C_{17} saturated fatty acid precursors for this reaction.

DISCUSSION

B. thuringiensis and *B. anthracis* both produce branched-chain fatty acids as major constituents of the total fatty acids (70 to 80%), and in this

TABLE 6. *Relative abundance of unsaturated fatty acids and their related saturated fatty acids of Bacillus thuringiensis grown on glucose-yeast extract medium (GY) or on Pennassay Broth (PB)*

Organism[a]	Medium	$i-C_{16}$ acids[b]			$i-C_{17}$ acids[b]			$a-C_{17}$ acids[b]			$n-C_{16}$ acids[b]		
		Saturated	Unsaturated	Ratio	Saturated	Unsaturated	Ratio	Saturated	Unsaturated	Ratio	Saturated	Unsaturated	Ratio
		%	%		%	%		%	%		%	%	
B. thuringiensis (I)	GY	8.1	5.4	0.7	5.5	2.7	0.5	1.9	1.4	0.7	1.7	13.2	7.8
	PB	7.2	3.4	0.5	8.7	2.6	0.3	2.7	0.1	0.0	3.4	12.7	3.7
B. thuringiensis (II)	GY[c]	10.1	6.9	0.7	5.9	3.3	0.6	3.7	2.7	0.7	1.5	11.0	7.3
	PB	5.1	3.6	0.7	10.1	5.5	0.5	5.3	1.9	0.4	3.6	11.2	3.1

[a] The organisms were grown for 16 hr at 37 C in 100 ml of either medium on a shaker. For the subsequent procedures used, see Table 4.
[b] See Table 2 for the abbreviations used for fatty acids.
[c] Taken from Table 4.

respect they are similar to the 10 species of the genus *Bacillus* previously studied (6). The nature and relative abundance of fatty acids produced by *B. thuringiensis* and *B. anthracis* are very similar to those of *B. cereus* [some fatty acids with less than 14 carbon atoms, more $i-C_{15}$ acid than $a-C_{15}$ acid, and significant proportions (14 to 20%) of unsaturated fatty acids] but are clearly distinguishable from *B. alvei, B. brevis, B. circulans, B. licheniformis, B. macerans, B. megaterium, B. polymyxa, B. pumilus,* and *B. subtilis* (no fatty acids with less than 14 carbon atoms, anteiso acid most abundant, and no unsaturated fatty acids; 6).

As has been discussed earlier, *B. thuringiensis* and *B. anthracis* are taxonomically closely related to *B. cereus*. The striking similarity in the nature and relative abundance of fatty acids produced by these three species is indicative of close similarity among them in biochemical activities involved in fatty acid metabolism. *B. thuringiensis, B. anthracis,* and *B. cereus* all produce fatty acids with fewer than 14 carbon atoms, n- and $i-C_{12}$, and i- and $a-C_{13}$, which are not significantly produced by the majority of the bacilli.

There are, however, minor but clear differences in the synthesis of these fatty acids between *B. thuringiensis* and *B. anthracis*. *B. thuringiensis*, like *B. cereus*, has higher relative proportions of $i-C_{12}$, $i-C_{13}$, and $a-C_{13}$ acids than does *B. anthracis*. *B. anthracis* is thus somewhat intermediate between *B. cereus* and the majority of the bacilli; probably the fatty acid synthesizing system of *B. anthracis* has properties transitional between those of *B. cereus* and the majority of the bacilli. Although these results were obtained by the study of only two strains each of *B. thuringiensis* and *B. anthracis*, the strains were obtained from different sources, and the measurements on the corresponding strains agreed with each other. Thus, the observed differences between the two species seem to be significant.

Burdon (1) found that virulent strains of *B. anthracis* could easily be distinguished from avirulent strains of *B. anthracis* and from *B. cereus* even without performing animal tests, although it was sometimes difficult to tell these latter two apart. Thus, for avirulent strains Burdon substantially agreed with the conclusion of Smith et al. (9), that *B. anthracis* is a variety of *B. cereus*. On the other hand, Leise et al. (7) found that *B. anthracis*, regardless of pathogenicity, could be differentiated from *B. cereus* by various tests, including bacteriophage susceptibility. Thus, they claimed that *B. anthracis* should not be considered as a variety of *B. cereus* but as a separate species. Our measurements have been on virulent strains of *B. anthracis*. In view of the reported differences between virulent and avirulent strains, it would be worthwhile to carry out similar analyses on avirulent strains.

There are marked differences in the relative abundances of fatty acids produced by the solid culture and the liquid cultures of *B. thuringiensis* II (Table 4). The solid culture of *B. thuringiensis* II gave relatively higher proportions of $i-C_{14}$, $i-C_{16}$, and $n-C_{16}$ acids and lower proportions of $i-C_{13}$, $i-C_{15}$, $a-C_{13}$, $a-C_{15}$, and $n-C_{16}^{1-}$ as compared with the liquid culture. Kaneda (5) has shown that the relative proportions of the three pairs of branched-chain fatty acids ($i-C_{14}$ and $-C_{16}$, $i-C_{15}$ and $i-C_{17}$, and $a-C_{15}$ and $a-C_{17}$) produced by *B. subtilis* are a function of the relative availability of precursors of the branched portions of these fatty acids, namely, either α-keto acids related to valine, leucine, and isoleucine, or the amino acids themselves, supplied endogenously or exogenously to the systems of the fatty acid synthesis. Since the two media used were of

the same composition except for the presence or absence of agar (2%), and since glucose was the major carbon source, these differences should be due to the differences in the activity of the systems synthesizing these precursors from glucose. Hence, the solid culture is probably more active in the synthesis of α-ketoisovalerate (keto acid related to valine) but less active in the syntheses of α-ketoisocaproate (keto acid related to leucine) and of α-keto-β-methylvalerate (keto acid related to isoleucine) than is the liquid culture.

There are also significant differences in the ratios of each unsaturated fatty acid to the corresponding saturated fatty acid produced by the agar and the submerged cultures (Table 4). This is most pronounced for the n-16-carbon acids. The sum of n-C_{16} plus n-$C_{16}^{1=}$ is almost the same for both cultures, but the relative proportion of the unsaturated acid is much higher for the submerged culture. In considering this observation, first recall the two alternative pathways in microorganisms for the biosynthesis of monounsaturated fatty acids. One includes the direct desaturation of a saturated long-chain fatty acid substrate to yield the corresponding monounsaturated fatty acid (2). The other pathway includes a medium-chain α-unsaturated fatty acid as an intermediate that is elongated to a C_{16} or C_{18} monounsaturated fatty acid. The first occurs commonly in aerobic organisms, and the second occurs commonly in anaerobic organisms. Members of the genus *Bacillus*, being aerobic organisms, are likely to produce monounsaturated fatty acids by direct desaturation of the corresponding fatty acids. Since this is an aerobic process, diffusion of oxygen to the growing cells may be a rate-determining factor limiting the conversion of saturated to unsaturated fatty acid, and this limitation is most likely to be observed in the n-C_{16} pair since n-C_{16} acid is the most effective precursor in desaturation. Thus, the probable slower rate of diffusion of oxygen to the bulk of culture in the solid medium as compared to the liquid medium (which is stirred by the shaker action) could explain the poor conversion of n-C_{16} acid to n-$C_{16}^{1=}$ acid in the solid medium.

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