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Fatty Acids in the Genus *Bacillus*

I. Iso- and Anteiso-Fatty Acids as Characteristic Constituents of Lipids in 10 Species[1]

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Fatty acids produced by 22 strains of 10 species of the genus *Bacillus* were analyzed on a very efficient and selective gas-liquid chromatographic column. All of the 10 species, *alvei*, *brevis*, *cereus*, *circulans*, *licheniformis*, *macerans*, *megaterium*, *polymyxa*, *pumilus*, and *subtilis*, produced eight fatty acids, six branched (anteiso-C_{15}, anteiso-C_{17}, iso-C_{14}, iso-C_{15}, iso-C_{16}, and iso-C_{17}) and two normal (n-C_{14} and n-C_{16}). In all cases, the six branched-chain fatty acids made up over 60% of the total fatty acids. In addition to the eight fatty acids, *B. cereus* produced four extra fatty acids, three branched (anteiso-C_{13}, iso-C_{12}, and iso-C_{13}) and one monoenoic-n-C_{16}. Furthermore, there were distinct differences in the relative amounts of fatty acids produced between *B. cereus* and the remaining nine species. *B. cereus* produced iso-C_{15} fatty acid in the largest amount on a glucose-yeast extract medium as well as on Pennassay Broth. On the other hand, for the remaining nine species, anteiso-C_{15} fatty acid was the major fatty acid from the glucose-yeast extract medium, whereas the amount of iso-C_{15} fatty acid from Penassay Broth became comparable to that of anteiso-C_{15} fatty acid. Mechanisms and various factors affecting the fatty acid distribution pattern in the 10 *Bacillus* species are discussed.

The major fatty acids produced by most living organisms are straight-chain acids with or without unsaturation in the carbon chain (myristic, palmitic, stearic, oleic, and linoleic acids). Branched-chain fatty acids having one methyl group at the penultimate (iso-) or the antepenultimate (anteiso-) positions are rare but occur as the major constituents of lipids in a limited number of organisms, mostly microorganisms (10), including *Bacillus subtilis* (4, 14), *B. megaterium* (16), and *B. cerecus* (11), which are all gram-positive bacteria.

Previous studies from this laboratory have examined the occurrence and biochemical mechanisms for the formation of branched-chain fatty acids in *B. subtilis*. The formation of branched-chain fatty acids has important relationships to the metabolism of amino acids. It is of interest to explore the generality of branched-chain fatty acids among other species in the genus *Bacillus*. The existence of chain branching and possible variations in the proportions of various branched-chain compounds may be significant as indicating different metabolic mechanisms, and may possibly be of some use in identification and taxonomy of bacterial species. This paper describes the results of an examination of 10 available *Bacillus* species.

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MATERIALS AND METHODS

Microorganisms. B. subtilis (ATCC 7059) was the same culture used in earlier work (4). All the remaining organisms were kindly supplied by Mary T. Clement, National Research Council of Canada, Ottawa (Table 1). All of the organisms were maintained as stock cultures on Nutrient Agar (Difco) slants.

Culture media. The standard medium contained 1% glucose, 0.1% yeast extract (Difco), and 1% (v/v) of the inorganic salt stock solutions I and II in distilled water (5). Other media used were Pennassay Broth (Difco), and Nutrient Agar (Difco).

Standard fatty acids. Branched-chain fatty acid samples chemically prepared in this laboratory (4) were used as standards. Fatty acids isolated from the lipids of *B. subtilis* (ATCC 7059) which have been identified in this laboratory by various chemical and physical properties, including gas-liquid chromatographic retention volumes on two columns (4), were also used as positional standards in gas-liquid chromatography. All other fatty acids used as references were commercial products.

Preparation of bacterial fatty acid samples. Stock cultures were transferred to the standard medium containing 2% agar (Difco) and were incubated at

TABLE 1. *Cultures of the genus Bacillus*[a]

NRC no.	Name	Original no.	Source	ATCC no.
B-32	*B. alvei*	USDA 685	N. R. Smith	10871
B-84	*B. alvei*	127	A. G. Lockhead	6344
B-33	*B. brevis*	USDA 604	N. R. Smith	8246
B-34	*B. brevis*	USDA 616	N. R. Smith	—
B-17	*B. cereus*	USDA 201	N. R. Smith	7064
B-19	*B. cereus*	USDA 305	N. R. Smith	—
B-82	*B. cereus* var. *mycoides*	USDA 306	N. R. Smith	6463
B-28	*B. circulans*	USDA 746	N. R. Smith	4530
B-29	*B. circulans* (mucoid type)	USDA 760	N. R. Smith	8384
B-49	*B. licheniformis*	—	T. Gibson	—
B-50	*B. licheniformis*	46	T. Gibson	—
B-40	*B. macerans*	USDA 888	N. R. Smith	8244
B-41	*B. macerans*	USDA 1093	N. R. Smith	843
B-15	*B. megaterium*	USDA 234	N. R. Smith	8245
B-78	*B. megaterium*	USDA 246	N. R. Smith	246
C-3(2)	*B. polymyxa*	—	NRC Ottawa	—
C-42(3) E13	*B. polymyxa*	—	NRC Ottawa	—
B-12	*B. pumilus*	USDA 236	N. R. Smith	6631
B-13	*B. pumilus*	USDA 233	N. R. Smith	—
B-4	*B. subtilis*	USDA 231	N. R. Smith	6633
B-770	*B. subtilis*		R. Gordon	6051
—	*B. subtilis*	USDA 352	N. R. Smith	7059

[a] NRC, National Research Council; ATCC, American Type Culture Collection; USDA, U.S. Department of Agriculture.

30 C overnight. One loop, 2 mm in diameter, of the fresh culture thus obtained was inoculated into 100 ml of the standard medium in a 500-ml Erlenmyer flask, which was incubated for 16 hr at 37 C on a rotary shaker. The cells were collected by refrigerated centrifugation and washed once with 0.85% NaCl solution. The saponification of the washed cells and the extraction of acidic components were performed as described earlier (7).

Gas-liquid chromatography. Two columns were used at 195 to 198 C, which consisted of standard copper tubing (⅛ inch by 20 ft) packed with 7% ethylene glycol adipate polymer (theoretical plates of about 12,000 measured with methyl palmitate) or 7% Apiezon L on Anakron ABS (60/80 mesh, a product of Analytical Engineering Laboratories, Inc., Hamden, Conn.). Nitrogen was used as the carrier gas with a flow rate of 2 to 4 ml/min. The bacterial fatty acid samples were esterified with diazomethane (15) prior to injection into the chromatographic system, Aerograph model 600 with flame ionization detector.

Growth. A Klett-Summerson colorimeter (no. 66 filter) was used to measure growth. When organisms showed over 250 units after 16 hr of incubation, they had attained the stationary phase of growth.

Gas-liquid chromatographic identification of fatty acids. The well-known linear relationship between the logarithm of the retention volume and the number of carbon atoms among homologous fatty acids was used to characterize bacterial fatty acids. Fatty acid bromination and hydrogenation procedures described by James (3) and Kaneshiro and Marr (9), respectively, were used.

RESULTS

Identification of bacterial fatty acids. Fatty acids isolated from 21 strains of 10 species of the genus *Bacillus*, namely, *alvei*, *brevis*, *cereus*, *circulans*, *licheniformis*, *macerans*, *megaterium*, *polymyxa*, *pumilus*, and *subtilis* were characterized by gas-liquid chromatographic retention volumes on two columns, adipate and Apiezon L; furthermore, the bacterial fatty acids were brominated or hydrogenated by two different methods, and the products were characterized on the two columns. Nine of the ten species investigated, i.e., *alvei*, *brevis*, *circulans*, *licheniformis*, *macerans*, *megaterium*, *polymyxa*, *pumilus*, and *subtilis*, produced only eight major fatty acids, six branched (anteiso-C_{15}, anteiso-C_{17}, iso-C_{14}, iso-C_{15}, iso-C_{18}, and iso-C_{17}) and two normal (n-C_{14} and n-C_{16}). No unsaturated, oxygenated, or cyclopropane fatty acids were detected in any of these organisms

B. cereus, however, produced four more fatty acids, three branched (anteiso-C_{13}, iso-C_{12}, and iso-C_{13}) and a monoenoic (n-C_{16}), in addition to the eight fatty acids found in the other organisms. Detailed studies of the fatty acids produced by *B. cereus* will be reported elsewhere.

Fatty acid distribution patterns of organisms grown on a glucose-yeast extract medium. Figures 1, 2, and 3 show gas-liquid chromatograms of bacterial fatty acid samples isolated from 10 species of the genus *Bacillus*. These species may be

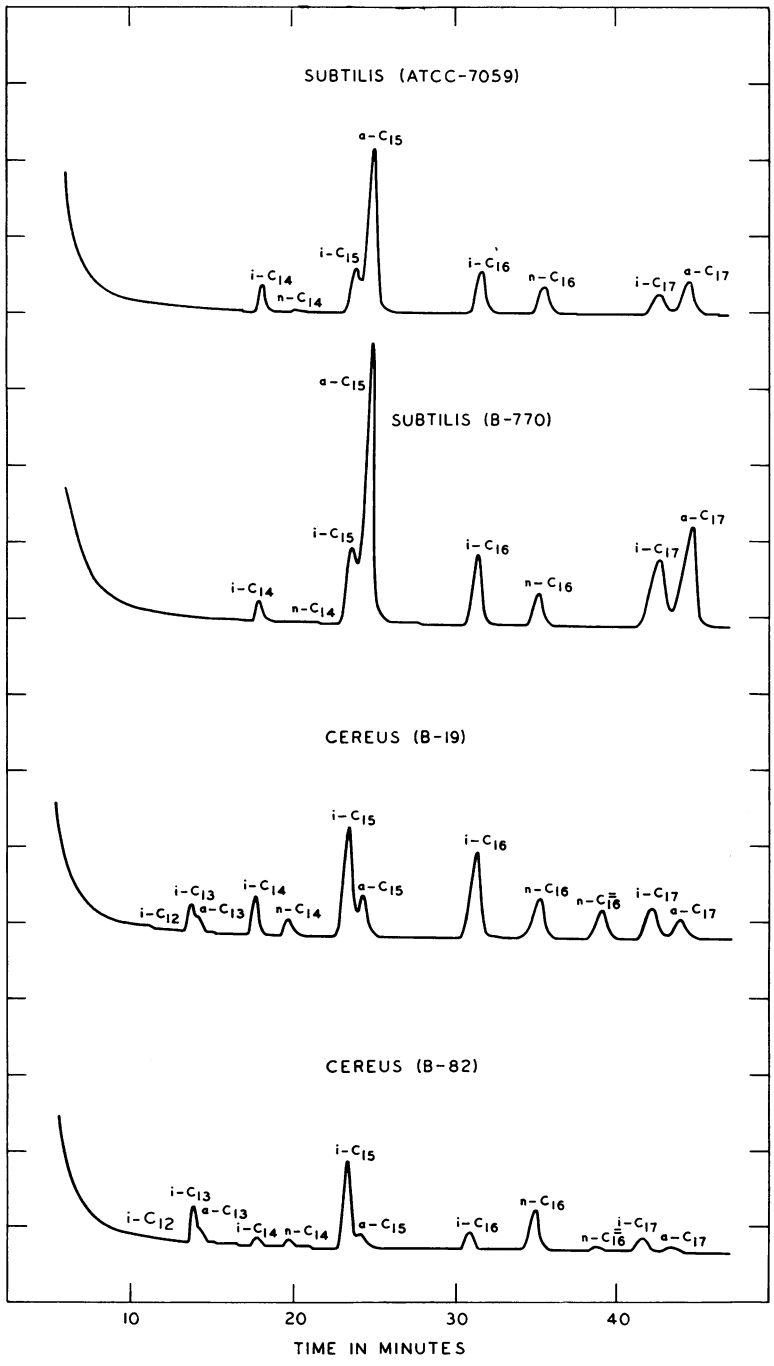

FIG. 1. *Gas-liquid chromatograms of bacterial fatty acids. The total fatty acids of a 100-ml culture of Bacillus subtilis (ATCC-7059), B. subtilis (B-770), B. cereus (B-19), and B. cereus var. mycoides (B-82) were 2.46, 3.33, 2.82, and 1.82 mg, respectively, on the basis of an internal standard, methyl pentadeanoate. In all cases, a portion of the fatty acid sample equivalent to 2 ml of the culture was injected.*

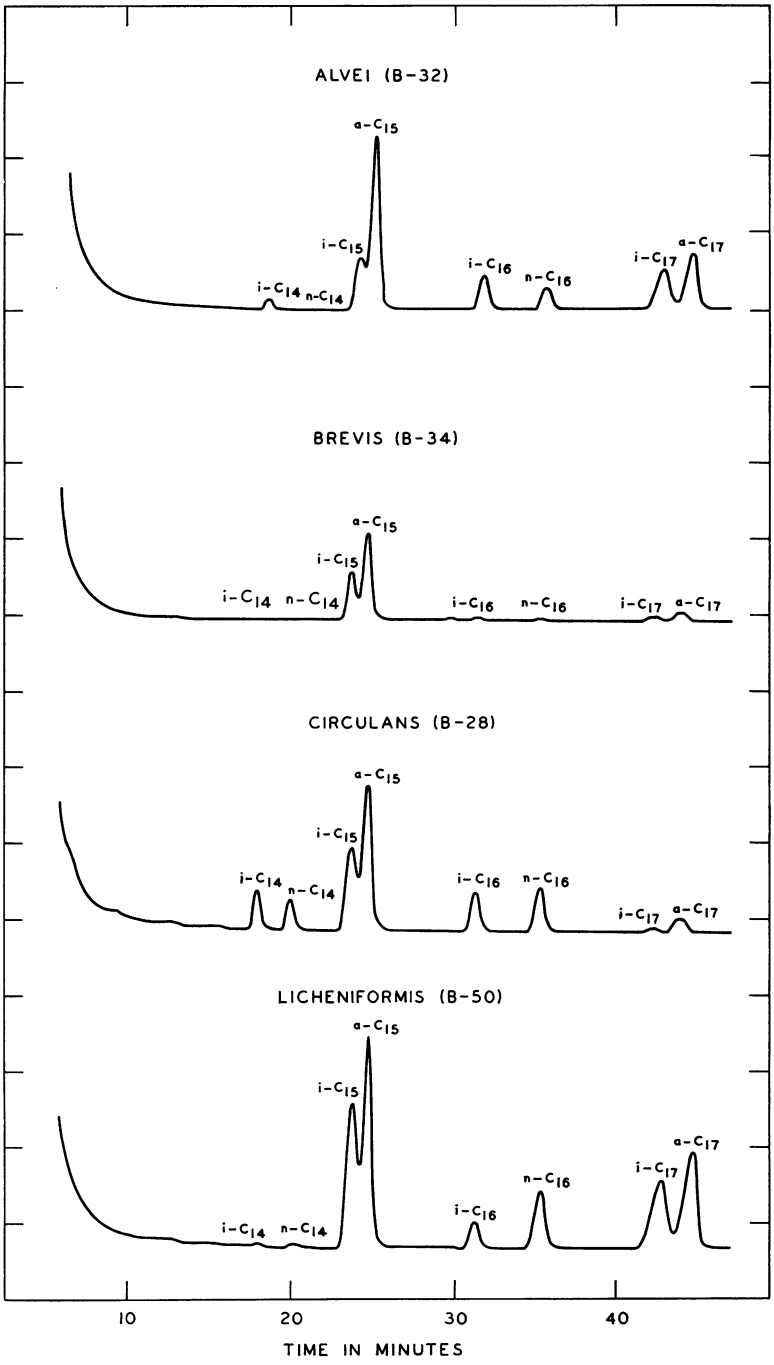

FIG. 2. *Gas-liquid chromatograms of bacterial fatty acids. The total fatty acids of a 100-ml culture of Bacillus alvei (B-32), B. brevis (B-34), B. circulans (B-28), and B. licheniformis (B-50) were 2.32, 0.35, 0.86, and 1.72 mg, respectively. Portions of the fatty acid samples equivalent to 2, 5, 5, and 4 ml, respectively, of the culture were injected.*

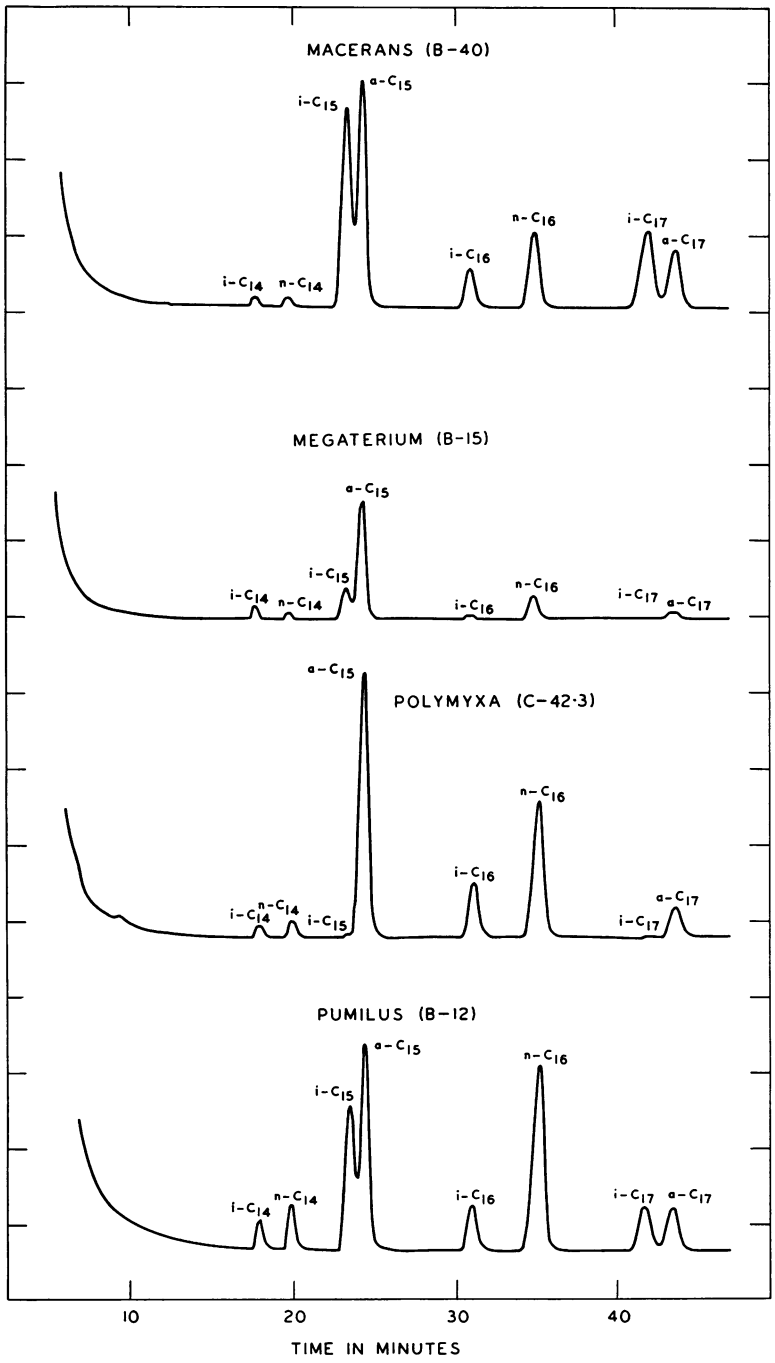

FIG. 3. *Gas-liquid chromatograms of bacterial fatty acids. Total fatty acids of a 100-ml culture of Bacillus macerans (B-40), B. megaterium (B-15), B. polymyxa (C-42.3), and B. pumilus (B-12) were 2.14, 0.89, 1.33, and 2.90 mg, respectively. Portions of the fatty acid samples, equivalent to 2, 2, 3, and 2 ml, respectively, of the culture were injected.*

divided into two groups. The first group included nine members, namely, *alvei*, *brevis*, *circulans*, *licheniformis*, *macerans*, *megaterium*, *polymyxa*, *pumilus*, and *subtilis*, all of which showed eight peaks on gas-liquid chromatograms. The second group consisted of only one member and its variety, namely, *B. cereus* and *B. cereus* var. *mycoides*, which showed eleven peaks, one of which was made up of two over-lapping peaks. Each chromatogram in Fig. 1 to 3 is shown at constant detector sensitivity, and under these conditions not all peaks were recorded. All peaks observed at a four times greater sensitivity are listed on the chromatograms.

Relative proportions of 4 pairs of fatty acids in 18 strains representing the 9 species in the first group are shown in Table 2. The values in the table are averages from triplicate experiments, the growth levels of which are also given in the table. The values from each experiment for the first two fatty acid pairs fell within ±5%, and those for the next two fatty acid pairs fell within ± 10%. Fatty acids of similar structure and differing only by 2-carbon units in chain length were considered, according to the biosynthetic mechanism discussed later, to come from the same precursor and, hence, were combined in Tables 2 to 5.

The combined anteiso-fatty acids (anteiso-C_{15} and anteiso-C_{17}) were the most abundant pair of acids in all the organisms, with the possible exception of *B. macerans* (B-40), and anteiso-C_{15} fatty acid was found to be the single most abundant fatty acid in all organisms. The odd-numbered iso-fatty acids (iso-C_{15} and iso-C_{17}) generally were next in order of abundance, and the even-numbered iso- (iso-C_{14} and iso-C_{16}) and normal (n-C_{14} and n-C_{16}) fatty acids were of low and variable abundance. Significant differences among the even-numbered fatty acids (iso- and normal) between two strains of the same species were observed in some cases (*B. alvei* and *B. brevis*) but not in others. These differences are not necessarily the results of differences in growth (compare *B. subtilis* and *B. megaterium* with *B. alvei* and *B. brevis*).

In contrast to the organisms mentioned above, the three strains of *B. cereus* produced odd-numbered iso-fatty acids in highest proportion and iso-C_{15} fatty acid in greatest amount (Table 3). *B. cereus* var. *mycoides* could be distinguished from the typical *B. cereus* by the higher proportions of normal fatty acids and of odd-numbered iso-fatty acids produced. As has already been pointed out, *B. cereus* also produces three branched-chain C_{12} or C_{13} acids and an unsaturated normal C_{16} acid.

Fatty acid distribution patterns of organisms grown on Pennassay Broth medium. Ten species of the genus *Bacillus* were grown on the amino acid-rich Pennassay Broth medium. In all cases,

TABLE 2. *Fatty acid distribution patterns of nine species of the genus Bacillus grown on a glucose-yeast extract medium*

Organism	Strain	Per cent fatty acids[a]				Growth (Klett units)		
		a-C_{15} + a-C_{17}	i-C_{15} + i-C_{17}	i-C_{14} + i-C_{16}	n-C_{14} + n-C_{16}	Expt 1	Expt 2	Expt 3
B. alvei	B-32	55	30	10	5	259	266	272
	B-84	45	37	3	16	~0	~0	119[b]
B. brevis	B-33	39	31	5	25	~0	~0	146[b]
	B-34	52	32	8	7	206	102	52
B. circulans	B-28	39	27	15	19	100	84	89
	B-29	35	27	5	33	254	238	246
B. licheniformis	B-49	49	41	3	7	139	126	135
	B-50	44	40	4	12	204	156	167
B. macerans	B-40	39	43	7	11	276	177	274
	B-41	47	39	4	10	157	169	136
B. megaterium	B-15	66	16	8	10	390	355	380
	B-78	55	28	5	12	190	188	191
B. polymyxa	C-3(2)	72	2	12	14	~0	200	146
	C-42(3)	61	3	9	28	112	202	189
B. pumilus	B-12	33	26	8	33	244	242	264
	B-13	38	28	3	21	189	197	256
B. subtilis	B-4	51	28	15	6	79	106	88
	B-770	58	25	11	6	254	256	286

[a] Average of three experiments with growth, as shown in columns on right.
[b] Growth after 24 hr of incubation.

TABLE 3. *Fatty acid distribution patterns of three strains of Bacillus cereus grown on a glucose-yeast extract medium*

Strain	Per cent fatty acids[a]					Growth (Klett units)		
	a-C_{13} + a-C_{15} + a-C_{17}	i-C_{13} + i-C_{15} + i-C_{17}	i-C_{13} + i-C_{14} + i-C_{16}	n-C_{14} + n-C_{16}	n-C_{16}^{-2}	Expt 1	Expt 2	Expt 3
B-17	22	33	27	12	6	287	292	268
B-19	22	37	24	11	6	355	320	256
B-82 (*mycoides*)	14	51	10	23	2	115	77	248

[a] Same as shown in Table 2.

with the exception of *B. polymyxa* and *B. cereus* var. *mycoides* (B-82), the synthesis of iso-C_{15} and iso-C_{17} fatty acids was increased remarkably over that obtained on the glucose-yeast extract medium; the two pairs of fatty acids, anteiso-C_{15} and anteiso-C_{17}, and iso-C_{15} and iso-C_{17}, together made up over 80% of the total fatty acids (Table 4). The amount of n-C_{14} and n-C_{16} fatty acids decreased to 2 to 8% of the total fatty acids.

B. polymyxa, however, produced anteiso-fatty acids in extremely high proportion, equivalent to that attained when the organism was grown on the standard medium. This may be related to the fact that the organism produces the antibiotic polymyxin which contains an anteiso-fatty acid as a component (17); very little iso-C_{15} and iso-C_{17} fatty acids were detected.

It is of interest that when the standard medium was replaced with Pennassay Broth medium the synthesis of iso-C_{15} and iso-C_{17} fatty acids by *B. cereus* (B-17 and B-19) was greatly increased, whereas the fatty acid distribution pattern of *B. cereus* var. *mycoides* (B-82) was unchanged (Table 5).

DISCUSSION

All 10 species and one variety of the genus *Bacillus* (a total of 22 strains) examined in this work have been shown to produce branched-chain fatty acids as major fatty acid constituents. In the cases of the other genera shown to have branched-chain fatty acids, *Micrococcus*, *Sarcina*, and *Staphylococcus*, only one species of each has been studied (10).

Based on the work of Horning et al. (2), it is probable that the distinctive aspect of the biosynthesis of branched-chain fatty acids is the formation of branched-chain precursors. These authors were able to demonstrate the synthesis of branched-chain fatty acids by a partially purified enzyme system of mammalian tissue primarily responsible for the synthesis of myristic and palmitic acids, if the appropriate branched-chain acyl coenzyme A (CoA) substrate were supplied to the system. A similar mechanism for the synthesis of branched-chain fatty acids has been found in *B. subtilis*. In this case, however, α-methylbutyryl CoA, the initiator of the synthesis of anteiso-C_{15} and anteiso-C_{17} fatty acids, is synthesized from L-α-keto-β-methyl-valerate, i.e., the α-keto acid corresponding to L-isoleucine (8). Similarly, isovaleryl CoA and isobutyryl CoA are synthesized from the α-keto acids corresponding to L-leucine and L-valine, respectively (5, 6). This key step to provide the necessary precursor must depend on an enzyme system, α-keto acid dehydrogenase. Subsequently, a C_2 precursor, presumably malonyl CoA, is repeatedly condensed with α-methylbutyryl CoA (or one of the other CoA esters) to elongate the chain length to 15 or 17 carbon atoms.

On the basis of the results quoted above for *B. subtilis*, we would expect all of the 10 species of *Bacillus* examined here to possess similar α-keto acid dehydrogenase enzyme systems whereby the appropriate branched, short-chain acyl CoA esters are produced. Preliminary experiments with cell-free extracts of *B. cereus* and *B. subtilis* have shown such an enzyme system to be present.

Although 10 *Bacillus* species probably contain this distinctive α-keto dehydrogenase, which leads to the synthesis of branched-chain fatty acids, differences in the fatty acid distribution patterns point to some secondary effects. Earlier work done in this laboratory (7) has shown that in *B. subtilis* the relative proportion of the three pairs of branched-chain fatty acids is a function of the relative availability of the three corresponding acyl CoA esters produced from two separate sources; firstly, from the three specific amino acids added to the culture medium as free acid, as peptide, or as protein and, secondly, by de novo synthesis from simple precursors derived from glucose. When the standard medium, an amino acid-poor medium, is used, the amino acids present in limited quantities are expected to be utilized mostly for the protein synthesis rather than for the branched-chain fatty acid synthesis

TABLE 4. *Fatty acid distribution patterns of nine species of the genus Bacillus grown on Pennassay Broth medium*

Organism	Strain	Per cent fatty acids[a]				Growth (Klett units)	
		a-C_{15} + a-C_{17}	i-C_{15} + i-C_{17}	i-C_{14} + i-C_{16}	n-C_{14} + n-C_{16}	Expt 1	Expt 2
B. alvei	B-32	59	35	3	3	170	180
	B-84	63	16	8	13	180	206[b]
B. brevis	B-33	42	35	16	8	49	26[b]
	B-34	37	54	7	2	242	284
B. circulans	B-28	53	32	6	9	98	104
	B-29	44	50	3	3	252	274
B. licheniformis	B-49	36	58	3	3	134	250
	B-50	49	48	1	2	184	165
B. macerans	B-40	39	56	2	3	192	202
	B-41	40	54	3	3	255	149
B. megaterium	B-15	55	35	4	5	169	150
	B-78	45	46	4	5	166	154
B. polymyxa	C-3(2)	73	4	20	8	59	102
	C-42(3)	74	3	13	10	59	138
B. pumilus	B-12	44	50	3	3	284	292
	B-13	45	46	4	5	152	187
B. subtilis	B-4	55	38	4	3	184	225
	B-770	52	40	5	3	278	282

[a] Average of two experiments with growth, as shown in columns on right.
[b] Growth after 24 hr of incubation.

TABLE 5. *Fatty acid distribution of three strains of Bacillus cereus grown on Pennassay Broth medium*

Strain	Per cent fatty acids[a]					Growth (Klett units)	
	a-C_{13} + a-C_{15} + a-C_{17}	i-C_{13} + i-C_{15} + i-C_{17}	i-C_{12} + i-C_{14} + i-C_{16}	n-C_{14} + n-C_{16}	n-C_{16}^{-2}	Expt 1	Expt 2
B-17	10	64	10	15	1	338	365
B-19	14	55	14	15	1	405	475
B-82 (*mycoides*)	15	52	9	22	2	135	117

[a] Same as shown in Table 4.

(8), and the source of these branched, short-chain acyl CoA esters would depend largely upon their de novo synthesis. In other words, the relative proportion of the three pairs of branched-chain fatty acids produced (which is measured by gas-liquid chromatography) is in the same order as the relative activity of the three systems producing α-methylbutyryl CoA, isovaleryl CoA, or isobutyryl CoA by de novo synthesis.

If an extremely large amount of the specific amino acid, L-isoleucine, L-leucine, or L-valine, is added to the culture medium, the synthesis of the structurally related pair of fatty acids is greatly increased (7). If all amino acids are present in abundance, for example, if Pennassay Broth is used, de novo synthesis of the three branched, short-chain acyl CoA esters is no longer the major rate-determining factor for the synthesis of branched-chain fatty acids; rather, the relative proportion of the three pairs of branched-chain fatty acids produced (which is measured by gas-liquid chromatography) depends largely upon the relative proportion of the specific three amino acids, L-isoleucine, L-leucine, and L-valine, supplied exogenously. Consequently, the fatty acid distribution pattern is less specific for each member of the genus *Bacillus*; this is shown experimentally.

The changes in the proportions of normal fatty acids when the standard medium is replaced by Pennassay Broth are of interest. Normal fatty acids occur in the least amounts in the majority of the 10 *Bacillus* species, and their proportions are even smaller when the Pennassay Broth is used

(Table 5). This decrease may be due to malonyl CoA, the chain extender in the fatty acid synthesis being available in only limited supply, so that the relative proportions of the various fatty acids depend on the relative proportions of the corresponding initiators; with a more plentiful supply of branched-chain amino acids, the total proportion of branched-chain fatty acids is correspondingly increased. The observation that the total amount of fatty acids per unit of growth was found to be relatively independent of which type of medium was used (see also reference 7) supports this hypothesis.

Two species, *B. polymyxa* and *B. cereus*, in 10 *Bacillus* species examined are found to have distinct fatty acid distribution patterns. The differences in *B. polymyxa* are quantitative: the proportions of anteiso-C_{15} and anteiso-C_{17} fatty acids are extremely small. However, the differences in *B. cereus* are both quantitative and qualitative: iso-C_{15} is the most abundant fatty acid instead of anteiso-C_{15}, and an extra four fatty acids are produced. Quantitative differences may arise for the reasons given above, but the production of additional fatty acids requires further consideration. An unsaturated fatty acid, monoenoic-n-C_{16}, is found only in *B. cereus*. Preliminary experiments indicate that this difference may only be qualitative rather than quantitative. By use of a silver nitrate-impregnated silicic acid column for preliminary concentration by column chromatography, small amounts of unsaturated fatty acids were found in *B. subtilis*. Possibly, the remaining species may be able to produce similar small amounts of unsaturated fatty acids. In addition, *B. megaterium* has been found to produce unsaturated fatty acids (1).

Fatty acids having chain lengths shorter than 14 carbon atoms are also found only in *B. cereus*. As has been discussed earlier, all the saturated fatty acids (branched-chain and normal) are synthesized by a single enzyme system from the appropriate acyl CoA ester related to the product fatty acids. Therefore, the enzyme system of *B. cereus* must be responsible for this difference. Three possibilities may be considered. (i) *B. cereus* has entirely different fatty acid-synthesizing enzyme proteins which release a product at an earlier stage of chain elongation. (ii) The enzyme system is no different from the others, but cofactors involved in the synthesis are different and favor the synthesis of shorter chain fatty acids. (iii) Physiological conditions in which the synthesis takes place are more favorable for the shorter-chain fatty acids. It would be desirable to determine which of these three possible mechanisms is actually involved.

An apparent contradiction to the general occurrence of branched-chain fatty acids throughout the genus *Bacillus* is given by "*Bacillus Alcaligenes*" and "*Bacillus fluorescens*" which have been reported to produce palmitic acid and monoenoic-C_{16} and -C_{18} fatty acids predominantly (13). These, however, are both gram-negative organisms which do not correctly belong to the genus *Bacillus*; the former is a member of the genus *Alcaligenes* and the latter is a *Pseudomonas* (*Bergey's Manual*). Consequently, the occurrence of straight-chain fatty acids and the absence of branched-chain fatty acids in these organisms are fully consistent with the results of this paper.

Although only 10 of 25 species of *Bacillus* have been examined in this work, it seems probable that the occurrence of particular types of fatty acids in a microorganism may be a reliable identification of its genus. A further example is the presence of cyclopropyl fatty acids in *Lactobacillus* (12). In some cases, the detailed distribution patterns of fatty acids will have taxonomic value in the identification of species, but further investigations are necessary to explore this possibility.

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