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Nutritional Alteration of the Fatty Acid Composition of a Thermophilic *Bacillus* Species

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The fatty acid composition of a thermophilic *Bacillus* sp. was altered by the addition of isobutyrate, isovalerate, α-methylbutyrate, leucine, and isoleucine to the growth medium. With isobutyrate, 81% of the fatty acids had 16 carbon atoms and 79% were iso-fatty acids with an even number of carbon atoms. With leucine, 58% of the fatty acids had 15 carbon atoms and 86% were iso-fatty acids with an odd number of carbon atoms. With isoleucine, 72% of the fatty acids had 17 carbon atoms and 88% were anteiso-fatty acids with an odd number of carbon atoms. Thus, by altering the composition of the growth medium, cells were produced in which the majority of the fatty acids had either 15, 16, or 17 carbons and belonged to each of the three groups of branched-chain fatty acids. The wide variation observed in the fatty acid composition makes it unlikely that any specific branched-chain fatty acid is required for vital functions.

Branched-chain fatty acids are the principal fatty acids in members of the genus *Bacillus*, and in nearly all of the species examined, whether mesophilic, psychrophilic, or thermophilic, the most abundant fatty acid is either iso- or anteiso-pentadecanoic acid (1, 5, 9-12, 16-18, 20, 22). In one thermophilic species of *Bacillus*, however, iso-hexadecanoic acid was the predominant fatty acid when the organism was grown at lower temperatures and the predominant branched-chain fatty acid at all growth temperatures tested (3). This seemed to set this species apart from other members of the genus, and so environmental growth conditions under which other branched-chain fatty acids might be produced in the greatest quantity were investigated to determine more fully the extent of phenotypic variation of the branched-chain fatty acids.

Branched-chain fatty acids are synthesized by the successive elongation of the coenzyme A (CoA) thiolesters of short branched-chain acids (13). Those fatty acids common to most *Bacillus* sp. are conveniently divided into groups according to the terminal precursor; thus, acetyl-CoA, isobutyryl-CoA, isovaleryl-CoA, and α-methylbutyryl-CoA are the terminal precursors for normal fatty acids having an even number of carbon atoms (n-C_{2n}), iso-fatty acids having an even number of carbon atoms (i-C_{2n}), iso-fatty acids having an odd number of carbon atoms (i-C_{2n+1}), and anteiso-fatty acids having an odd number of carbon atoms (a-C_{2n+1}), respectively. The terminal precursors for the branched-chain fatty acids can be formed by the oxidative decarboxylation of the α-keto acids corresponding to valine, leucine, and isoleucine, and the addition of short branched-chain acids or branched-chain amino acids to the growth medium influences the fatty acid composition (6-8, 14, 17). This paper describes the fatty acid composition of a thermophilic *Bacillus* sp. grown in the presence of various branched-chain organic acids.

MATERIALS AND METHODS

A description of the organism and conditions for its maintenance have been reported previously (2). Cultures for fatty acid analysis were grown in Fernbach flasks at 50 C (3) in medium containing 1% acetate as a carbon source (2), to which various branched-chain acids were individually added to a final concentration of 1%. Growth studies were conducted with 50-ml cultures in 125-ml Erlenmeyer flasks at 50 C. Isobutyric acid, isovaleric acid, DL-2-methylbutyric acid, and L-(+)-isoleucine were obtained from Eastman Kodak Co., Rochester, N.Y., and L-leucine and L-valine were from Nutritional Biochemicals Corp., Cleveland, Ohio. Procedures for harvesting the cells, extracting the lipids, and preparing the methyl esters of the fatty acids have been described (3). The groups of fatty acids were considered without regard for the degree of unsaturation. To eliminate any possible interference by the unsaturated fatty acids, the fatty

acid methyl esters were hydrogenated in hexane with a 5% palladium-on-charcoal catalyst to convert the unsaturated esters to their saturated counterparts, even though earlier work had established that in organisms grown at 50 C less than 3% of the fatty acids are unsaturated (3). The fatty acid esters were then analyzed by gas-liquid chromatography on a column of 15% EGSS-X on Gas Chrom P (100 to 120 mesh) (Applied Science Laboratories, Inc., State College, Pa.) at 164 C; other conditions have been described (3). The fatty acid composition was calculated from the areas of the chromatographic peaks, which were determined by triangulation.

RESULTS AND DISCUSSION

The organism used in this study can use acetate as well as glucose as a carbon source. Other organic acids were tested to see whether they could serve as carbon sources. However, neither the branched-chain acids, isobutyric, isovaleric, and α-methylbutyric acids, nor the amino acids, valine, leucine, and isoleucine, could replace acetate as a carbon source in the growth medium. When they were added singly to the acetate medium, the organism grew in all of the resulting media except the one containing valine. The growth rate was also retarded when isoleucine was present; however, leucine stimulated growth (Fig. 1). The inhibition of growth by valine and isoleucine has been reported for other organisms (e.g., 4) and is accommodated by the known biosynthetic pathways for the branched-chain amino acids and their regulation (15, 19). The means by which added leucine stimulated growth is not clear.

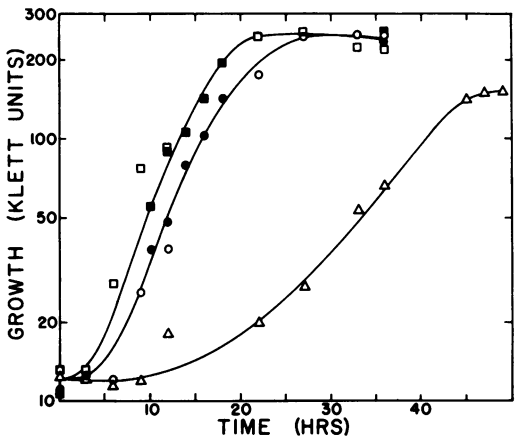

FIG. 1. *Effect of added leucine and isoleucine on bacterial growth. Turbidity was determined with a Klett-Summerson colorimeter, no. 66 filter, versus a distilled water blank. Symbols: ○ and ●, acetate medium (two experiments); □ and ■, acetate medium plus leucine (two experiments); △, acetate medium plus isoleucine. Each data point is the average of two identical flasks, and the individual values were always within 5% of the average value.*

The fatty acid composition of lipid extracts from cells grown in the different media is shown in Table 1. These data show that the fatty acid composition was considerably altered by the addition of the organic acids, resulting in cells in which over 50% of the fatty acids had either 15, 16, or 17 carbon atoms or in which over 50% of the fatty acids belonged to each of the three groups of branched-chain fatty acids (Fig. 2). Since a previous report showed that n-16:0 comprised over 60% of the fatty acids in cells grown at 60 C with glucose as a carbon source (3), this organism apparently functions normally when the majority of its fatty acids belong to any one of the four major groups. The addition of any one of the five branched-chain compounds increased the total branched-chain fatty acids from 70 to about 90%, with the greatest increase in the group for which the terminal precursor is produced by the added compound (Fig. 2). The addition of either of the amino acids resulted in the production of one group of branched-chain fatty acids almost exclusively.

Although both isovalerate and leucine are directly converted to the terminal precursor for the i-C_{2n+1} group of branched-chain fatty acids, the resulting fatty acid compositions are quite

TABLE 1. *Effect of organic acids in the growth medium on the fatty acid composition*

Fatty acid	Amount (weight percent)					
	None	Isobutyrate[a]	Isovalerate	α-Methylbutyrate	Leucine	Isoleucine
n-12:0	0.1	0.1	0.1	0.1	0.1	TR[b]
i-14:0	0.9	0.7	0.6	0.4	0.1	0.1
n-14:0	2.0	0.2	0.5	0.6	0.9	0.8
i-15:0	6.1	1.9	7.5	4.4	57.5	
a-15:0	4.9	0.6	3.4	5.8		15.7
n-15:0	0.3	0.1	0.3	0.4	0.1	0.1
i-16:0	30.3	75.6	36.4	19.4	1.4	1.4
n-16:0	26.9	5.5	9.7	7.9	8.7	8.9
i-17:0	5.6	4.8	11.5	6.8	28.6	
a-17:0	21.3	6.9	27.2	51.6	2.1[c]	71.8
n-17:0	0.2	0.1	0.3	0.3	0.1	0.2
i-18:0	0.6	3.0	1.2	0.6	TR	TR
n-18:0	0.9	0.6	0.8	1.0	0.4	0.8
i-19:0	TR	TR	0.1	0.1	0.1	
a-19:0	0.1	0.1	0.2	0.4		0.2

[a] Compound added to acetate growth medium.
[b] TR, Trace (<0.05%).
[c] This was observed as a small shoulder on the trailing edge of a large peak and the amount is only approximate.

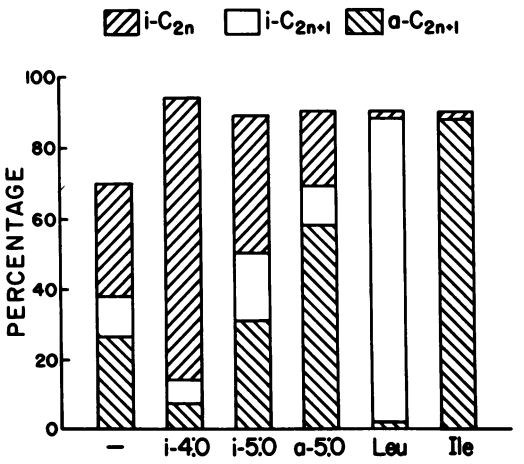

FIG. 2. *Influence of branched-chain compounds in the growth medium on the branched-chain fatty acid composition.*

different. The same situation was found for the pair α-methylbutyrate and isoleucine. The difference in the effects produced by the short branched-chain acids and the amino acids is more clearly seen when the ratios of each group of branched-chain fatty acids to normal fatty acids are compared (Table 2). The addition of any one of the three short branched-chain acids increased the relative amount of all three groups of branched-chain fatty acids; the greatest increase was in the group for which the added acid gave rise to the terminal precursor. Thus, addition of isobutyrate, isovalerate, and α-methylbutyrate produced relative increases of 11.3-, 4.2-, and 6.7-times in i-C_{2n}, i-C_{2n+1}, and a-C_{2n+1} fatty acids, respectively. The addition of leucine and isoleucine, on the other hand, increased the relative amounts of i-C_{2n+1} and a-C_{2n+1} fatty acids, respectively, but decreased the relative amounts of the other two groups of branched-chain fatty acids.

These results are similar to those for *B. subtilis* and are generally consistent with the scheme for branched-chain fatty acid biosynthesis proposed by Kaneda (8), according to which the α-keto acid analogues of the branched-chain amino acids compete in the rate-limiting, oxidative decarboxylation reaction, and the addition of one amino acid would thus increase one group of branched-chain fatty acids while decreasing the other two groups. The addition of a short branched-chain acid would not compete in the rate-limiting step, and so it would increase one group of branched-chain fatty acids while leaving the other two groups unaffected. The normal fatty acids were decreased in *B. subtilis*, however, presumably through the utilization of acetyl-CoA in the formation of the CoA thiolester of the added acid (8).

The fatty acid compositions resulting when leucine and isoleucine were added are strikingly similar (viz., 86% of the fatty acids were i-C_{2n+1} and 88% were a-C_{2n+1} when leucine and isoleucine were added, respectively; also the relative amounts of i-16:0 and n-16:0 are the same whether leucine or isoleucine was added), which suggests that the amino acids influence fatty acid biosynthesis in the same manner, such as that proposed above. The relative increases in all three groups of branched-chain fatty acids resulting from the addition of a short branched-chain acid cannot be entirely due to a decrease in the amount of normal fatty acids, however, because the relative increases in the two groups for which the added acid was not a terminal precursor were not the same when isobutyrate or α-methylbutyrate was added. The stimulation of growth by added leucine suggests that leucine biosynthesis is limiting in the absence of added leucine. The observation that i-C_{2n+1} fatty acids were the least abundant of the groups of branched-chain fatty acids also suggests that α-ketoisocaproate is preferentially transaminated to leucine rather than being used for fatty acid synthesis, and is consistent with Kaneda's postulate that the relative availability of terminal precursors determines the fatty acid composition (8, 9, 11).

The enzyme system responsible for chain elongation has a preference for producing branched-chain fatty acids with a methyl group at carbon 14. This is shown by the relatively specific production of i-16:0 when isobutyrate was present and a-17:0 when α-methylbutyrate

TABLE 2. *Effect of organic acids in the growth medium on the relative amounts of branched-chain fatty acid groups*

Fatty acid	Compound added to the acetate growth medium					
	None	Isobutyrate	Isovalerate	α-Methylbutyrate	Leucine	Isoleucine
i-C_{2n}/n-C_{2n}	1.1[a]	12.4	3.4	2.1	0.15	0.14
i-C_{2n+1}/n-C_{2n}	0.4	1.0	1.7	1.2	8.5	0
a-C_{2n+1}/n-C_{2n}	0.9	1.2	2.8	6.0	0.21	8.4

[a] Ratio.

or isoleucine was present. When elongation cannot produce a branched-chain fatty acid with a methyl group at carbon 14, as in the i-C_{2n+1} series, then fatty acids with methyl groups at either carbon 13 (i-15:0) or carbon 15 (i-17:0) are produced in roughly equal amounts.

The isolation of a mutant of *B. subtilis* that requires branched-chain fatty acids or their precursors for growth (21) demonstrates the essential nature of these compounds. But the extent of the variation in fatty acid composition which is tolerated by this organism makes it unlikely that specific branched-chain fatty acids are required for any vital function. A similar conclusion has been reached for sporulation in *B. megaterium* (17).

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