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Fatty Acids in *Bacillus larvae*, *Bacillus lentimorbus*, and *Bacillus popilliae*[1]

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The types of fatty acids produced by two strains each of *Bacillus larvae*, *B. lentimorbus*, and *B. popilliae*, and their distribution patterns, were studied by gas-liquid chromatography. All six organisms produced eight major fatty acids: six branched (iso-C_{14}, -C_{15}, -C_{16}, and -C_{17}, and anteiso-C_{15} and -C_{17}), two normal (n-C_{14} and -C_{16}), and two minor (n-C_{15} and monounsaturated n-C_{16}). In addition, some other trace acids were produced. Branched-chain fatty acids accounted for 54 to 85% of the total fatty acids. These compositions are similar to those previously found with 26 strains of 12 species of the genus *Bacillus*. Thus, an abundance of branched-chain fatty acids seems to be a characteristic of the biochemical nature of the genus *Bacillus*. It is noteworthy that marked differences between the nutritional requirements of the three insect pathogens used in the present study and those of the other 12 species of the genus *Bacillus* studied previously are not significantly reflected in their fatty acid composition.

Previous work (7, 8) has shown that 26 strains representing 12 species of the genus *Bacillus* all produce branched-chain fatty acids of the iso and anteiso series, with 14 to 17 carbon atoms, as the major components of the total fatty acids (60 to 80%). These branched-chain fatty acids in the organisms were further increased when the organisms were grown on an appropriate amino acid-rich medium. Species of the genus *Bacillus* pathogenic to insects, such as *B. thuringiensis*, and to mammals, such as *B. anthracis*, were found to have no major differences in fatty acids from the aforementioned species and, in particular, from *B. cereus*, either in the large proportion of branched-chain fatty acids or in the higher concentration of i-C_{15} acid as compared with a-C_{15} acid (8).

The work has now been extended to three other pathogenic species of *Bacillus*: *B. larvae*, *B. lentimorbus*, and *B. popilliae*. These organisms, all of which attack certain insects causing milky disease, have a rather different physiological nature than those previously studied. In particular, they do not grow on a simple medium containing glucose as the major carbon source. Indeed, growth of *B. popilliae*, and possibly of *B. lentimorbus*, on the chemically defined medium is stimulated by barbituric acid (10). Even on a complex medium, growth of these organisms is very scanty and they tend to lose viability in a short time (1). The purpose of the present study was to investigate whether this distinctive pathogenic behavior and these unusual growth requirements are reflected in the type and distribution of fatty acids in these insect pathogens as compared with other *Bacillus* species.

MATERIALS AND METHODS

Microorganisms. Two strains each of *B. larvae*, *B. lentimorbus*, and *B. popilliae* were kindly supplied by William C. Haynes, Northern Regional Research Laboratory, Peoria, Ill. Sources of isolates and original donors for *B. lentimorbus* B-2522 and B-2530, and for *B. popilliae* B-2309$_s$ and B-2519, were described by Haynes et al. (1). All cultures were maintained as stock cultures on 1.5% yeast extract, 0.2% glucose, and 0.6% K_2HPO_4 slants (10). Regular transfers at 5-day intervals were found to be essential.

Culture media. The following liquid culture media were employed: a medium composed of 1% glucose, 0.1% yeast extract (Difco), and inorganic salts (7); a medium containing 1.5% yeast extract, 0.2% glucose, and 0.6% K_2HPO_4; Heart Infusion Broth (BBL); Trypticase Soy Broth (BBL); and Penassay Broth (Difco).

Incubation. The organisms were grown in 100 ml of the appropriate medium in 500-ml Erlenmeyer flasks and were incubated at 30 C on a rotary shaker until such time as sufficient growth was observed. Cell density was determined by use of a Klett-Summerson colorimeter with a no. 66 filter (8).

Isolation of fatty acids and their identification by

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gas-liquid chromatography have been described in a previous paper (7).

RESULTS

Growth of B. larvae, B. lentimorbus, and B. popilliae. The yeast extract-glucose-phosphate medium (10) was found to be satisfactory for the growth of these organisms, and was used throughout this work. On this medium, the organisms showed an initial growth after 2 days of incubation and reached a stationary phase of growth in 5 to 8 days. The four other media tested were found to be unsatisfactory, as they gave either no growth or inconsistent growth.

Identification of bacterial fatty acids. Bacterial fatty acids were extracted from the organisms that had been grown on the standard medium (7). Co-chromatography of the original samples, and of the hydrogenated or the brominated samples, of these fatty acids against the standard fatty acids was carried out on the two columns, ethylene glycol adipate and SE-30, to identify each gas-liquid chromatographic peak component (8). The 10 major components thus identified were i-C_{14}, n-C_{14}, i-C_{15}, a-C_{15}, n-C_{15}, i-C_{16}, n-C_{16}, i-C_{17}, a-C_{17}, and monoenoic-n-C_{16} acids; some of the minor acids were i-C_{12}, i-C_{13}, and a-C_{13} acids (i-, n-, and a- represent iso, normal, and anteiso, respectively).

Fatty acid composition. The gas-liquid chromatograms shown in Fig. 1 illustrate the fatty acid composition of one strain of each species. The other strain in each species gave an almost indentical chromatogram. It is noteworthy that all three chromatograms are composed of the same peak components although, as can be seen in Fig. 1, the relative heights of the components vary with each chromatogram. More detailed accounts of the fatty acid composition are given in Tables 1, 2, and 3, in which the values represent the percentage of the total fatty acids made up of each major fatty acid and of each pair of fatty acids; the ratio of the two members of each pair of fatty acids is also shown. The reason for grouping two fatty acids with identical terminal chemical structure but with a two-carbon difference of chain length is based on the close relationship in the biosynthesis of the paired acids (2, 3).

All six organisms produced branched-chain fatty acids as the major component of the total fatty acids (54 to 85%), and the combined anteiso fatty acids (a-C_{15} + a-C_{17}) were in the largest proportion (46 to 68%) among the four pairs of the fatty acids. Generally, the order of decreasing abundance is as follows: combined anteiso acids, combined normal acids, combined iso odd-numbered acids, and combined iso even-numbered acids. Anteiso-C_{15} acid was, in all cases, produced in the largest proportion among individual fatty acids (39 to 69%). Second in abundance in *B. lentimorbus* and *B. popilliae* was n-C_{16} acid (palmitic acid), whereas *B. larvae* produced slightly more a-C_{17} acid than n-C_{16} acid. The remaining fatty acid components were present in much smaller proportions, at most 9%, and differences in their relative abundance were small. The order of abundance of these minor components was found to vary with each strain.

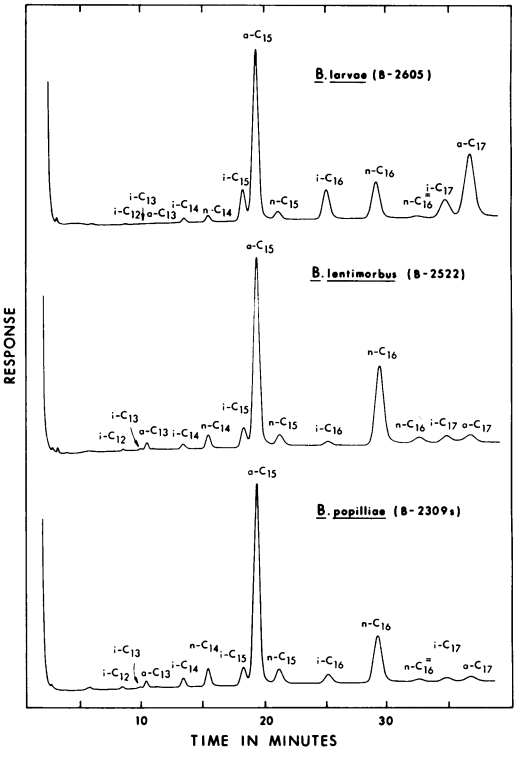

FIG. 1. *Gas-liquid chromatograms of the total fatty acids isolated from B. larvae, B. lentimorbus, and B. popilliae. Cells were grown on 100 ml of yeast extract-glucose medium for 104 hr at 30 C on a rotary shaker. B. larvae equivalent to 75 mg of dry cells (149 Klett units) and 1/150 of the total fatty acids was used. B. lentimorbus and B. popilliae were equivalent to 26 and 48 mg of dry cells (Klett units of 52 and 96), re-were used in each case. The column was 20 ft by 1/8 inch spectively, and 1/50 and 1/100 of the total fatty acids (610 cm by 0.3 cm), packed with ethylene glycol adipate (7%) on Anakron ABS 60/80 mesh (Analab, Inc., Hamden, Conn.), and was operated isothermally at 170 C (7).*

DISCUSSION

B. larvae, *B. lentimorbus*, and *B. popilliae* have a fatty acid composition basically similar to that

TABLE 1. *Fatty acid distribution patterns of two strains of Bacillus larvae*

Fatty acid	*B. larvae* B-2605[a]			*B. larvae* B-2610[a]		
	Amt of fatty acid[b]		C_n/C_{n+2}[c]	Amt of fatty acid[b]		C_n/C_{n+2}[c]
	Individual	Pair		Individual	Pair	
n-C_{14}	1.1			1.0		
n-C_{16}	11.5	12.6	0.10	12.2	13.2	0.08
i-C_{14}	0.8			0.0		
i-C_{16}	7.6	8.4	0.11	3.1	3.1	—
i-C_{15}	6.6			8.3		
i-C_{17}	6.6	13.2	1.00	4.2	12.5	1.98
a-C_{15}	38.7			50.2		
a-C_{17}	25.0	63.7	1.55	18.1	68.3	2.77

[a] Incubated on 100 ml of the yeast-glucose-phosphate medium in a 500-ml Erlenmeyer flask at 30 C; B-2605 for 104 hr yielding 75 mg of dry cells, and B-2610 for 222 hr yielding 88 mg of dry cells.

[b] Expressed as percentage of the total fatty acids.

[c] Ratio of first member of pair to second.

TABLE 2. *Fatty acid distribution patterns of two strains of Bacillus lentimorbus*

Fatty acid	*B. lentimorbus* B-2522[a]			*B. lentimorbus* B-2530[a]		
	Amt of fatty acid[b]		C_n/C_{n+2}[c]	Amt of fatty acid[b]		C_n/C_{n+2}[c]
	Individual	Pair		Individual	Pair	
n-C_{14}	5.3			1.3		
n-C_{16}	26.6	31.9	0.20	32.5	33.8	0.04
i-C_{14}	0.0			0.0		
i-C_{16}	0.6	0.6	—	1.4	1.4	—
i-C_{15}	5.9			2.4		
i-C_{17}	1.9	7.8	3.11	4.8	7.2	0.50
a-C_{15}	44.6			40.7		
a-C_{17}	1.5	46.1	29.7	7.3	48.0	5.57

[a] Incubated on 100 ml of the yeast-glucose-phosphate medium in a 500-ml Erlenmeyer flask at 30 C; B-2522 and B-2530 for 71 hr, yeilding 25 and 36 mg of dry cells, respectively.

[b] Expressed as percentage of the total fatty acids.

[c] Ratio of first member of pair to second.

TABLE 3. *Fatty acid distribution patterns of two strains of Bacillus popilliae*

Fatty acid	*B. popilliae* B-2309$_s$[a]			*B. popilliae* B-2519[a]		
	Amt of fatty acid[b]		C_n/C_{n+2}[c]	Amt of fatty acid[b]		C_n/C_{n+2}[c]
	Individual	Pair		Individual	Pair	
n-C_{14}	3.9			4.8		
n-C_{16}	18.8	22.7	0.21	9.8	14.6	0.49
i-C_{14}	1.8			3.5		
i-C_{16}	3.0	4.8	0.60	3.8	7.3	0.92
i-C_{15}	5.2			5.1		
i-C_{17}	1.6	6.8	3.25	1.0	6.1	5.10
a-C_{15}	56.5			62.0		
a-C_{17}	2.2	58.7	27.2	1.8	63.8	34.4

[a] Incubated on 100 ml of the yeast-glucose-phosphate medium in a 500-ml Erlenmeyer flask at 30 C; B-2309$_s$ and B-2519 for 104 hr, yielding 47 and 26 mg of dry cells, respectively.

[b] Expressed as percentage of the total fatty acids.

[c] Ratio of first member of pair to second.

of the 12 species of *Bacillus* previously studied. Branched-chain fatty acids are predominant, and a-C_{15} acid is the single fatty acid present in largest proportion in all species except for the three closely related species (8) *B. anthracis*, *B cereus*, and *B. thuringiensis*, in which i-C_{15} acid is most abundant.

As has been discussed in previous papers (5, 7), and on the basis of observations reported by Kaneda (3, 4, 6), Lennarz (9), and Horning et al. (2), branched-chain fatty acids are very likely to be produced in microorganisms by a mechanism similar to that involved in the synthesis of normal fatty acids (11): the terminal precursors isobutyryl, isovaleryl, and α-methylbutyryl coenzyme A (CoA) esters are elongated by repeated condensation of malonyl CoA to give the corresponding branched-chain fatty acids with 14 to 17 carbon atoms. According to this postulated mechanism, the relative proportion of the four pairs of fatty acids is a function of the relative availability to the fatty acid-synthesizing system of the four terminal precursors—acetyl CoA, isobutyryl CoA, isovaleryl CoA, and α-methylbutyryl CoA. In most species, the system producing α-methylbutyryl CoA appears to be most active. These characteristics of *Bacillus* are obviously more fundamental to its metabolism than are the characteristic differences among species,

such as the distinctive pathogenic behavior and the unusual growth requirements.

Within any pair of homologous fatty acids, differences in the relative amounts of the two members reflect different degrees of chain extension. In the present results, marked differences were found, particularly with the anteiso fatty acids (Tables 1, 2, and 3), both among the three species and sometimes between the two strains of a given species. Factors affecting the ratio are not well understood and work on this subject is in progress.

Of the 25 generally accepted species of *Bacillus* (*Bergey's Manual*), 15 representative ones have been examined for fatty acid composition. Branched-chain fatty acids predominate in all cases, so that it is now reasonable to conclude that this is a characteristic feature of the genus *Bacillus*, regardless of the wide differences observed in the physiological nature of the various species.

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