JOURNAL OF BACTERIOLOGY, Jan. 1972, p. 443-446

Vol. 109, No. 1
Printed in U.S.A.

Effect of Temperature on the Fatty Acid Composition of the Extreme Thermophiles, *Bacillus caldolyticus* and *Bacillus caldotenax*

A. WEERKAMP AND W. HEINEN

Department of Exobiology, Faculty of Science, University of Nijmegen, Nijmegen, The Netherlands

Received for publication 12 October 1971

Gas chromatographic analysis of extracts from *Bacillus caldolyticus* and *Bacillus caldotenax* grown at 60, 70, or 80 C showed that both contain branched-chain fatty acids as major constituents at all temperatures tested. With increasing temperature, a decrease of i-C15 and an increase of i-C17 fatty acids were observed in both strains, as well as a decrease of i-C16 fatty acids corresponding to an increase of n-C16 fatty acids. The most obvious difference was that the shifts observed with *B. caldolyticus* occurred mainly upon raising the temperature to 10 C above, and in *B. caldotenax* upon lowering the temperature to 20 C below, the optimal growth temperature.

The phenomenon of thermostability of microorganisms represents an intricate problem involving many factors that could be responsible for the stability of entire cells and cellular components. Presumably, the properties of many cellular constituents contribute simultaneously their individual share, and one of the possibilities is a specific structural composition of the membrane to prevent leakage at higher temperatures. The melting point of the major membrane constituents can in this context be regarded as a factor influencing the flexibility and stability of the membrane.

Comparative studies on the fatty acid composition of mesophilic and thermophilic bacteria have shown that a certain preference for the synthesis of elongated and branched fatty acids exists at higher temperature (1, 8). This is reflected from the changes occurring as a result of temperature alterations (1, 4). Since we found that one of our thermophilic strains responds in a typical manner to an increase in temperature from 50 to 80 C by the preferential synthesis of branched heptadecanoic acid and a total elimination of unsaturated fatty acids (4), a more detailed study seemed worthwhile. When the strains were grown at their optimal temperature, extracts from *Bacillus caldotenax* (at 80 C) showed four main peaks accounting for 87.6%, and *Bacillus caldolyticus* (at 70 C) showed five main compounds representing 88.5% of the total fatty acid fraction (Table 1).

Since we had previously found with *Thermus aquaticus* (4) that the relative amounts of individual fatty acids were greatly influenced by growth temperature, the strains were grown at 60, 70, or 80 C (Table 1). The most obvious responses to temperature increase are the decrease of i-C15 and the increase of i-C17 fatty acids. Another direct relation is given with the i-C16 plus n-C16 pair, with an increase of n-C16 and a decrease of i-C16 fatty acids upon raising of the temperature from 60 to 80 C. Bromination showed that oleic acid is the only unsaturated fatty acid present in the extracts at all temperatures tested.

The trends of the temperature-dependent alterations become quite clear if groups or pairs of the individual compounds, arranged in several ways, are compared (Table 2, Fig. 1). Comparing the changes which are similar in both strains, we find that the total amount of branched-chain fatty acids decreases with increasing temperature, whereas the straight-chain compounds follow the opposite direction. A distinct difference lies in the behavior of the odd-, and even-numbered compounds (Table 2, c through f). The branched and straight, odd fatty acids show only minor changes, whereas the branched, even compounds decrease and the straight, even compounds increase. The tendency to synthesize longer chains at higher temperatures is indicated by the enormous decrease of the a-C15 plus i-C15 fatty acid

TABLE 1. *Fatty acid composition of Bacillus caldolyticus and Bacillus caldotenax at different growth temperatures*[a]

Peak no.[b]	Fatty acid	*B. caldolyticus*			*B. caldotenax*		
		60 C	70 C	80 C	60 C	70 C	80 C
1	i-C13	0.3	0.3	0.3			
2	n-C13	0.2	0.2	0.2	0.1	0.3	0.1
3	i-C14	3.0	0.4	0.3	1.0	0.4	0.4
4	n-C14	1.5	1.3	0.9	1.0	0.8	1.0
5	i-C15	38.3	29.8	8.7	28.6	10.1	5.0
6	a-C15	0	0	1.2	1.9	2.2	1.9
7	n-C15	3.9	5.9	1.0	2.2	0.9	1.2
8	i-C16	20.0	11.8	7.9	27.2	16.6	8.5
9	n-C16	6.6	12.7	13.7	13.3	16.8	24.0
10	i-C17	17.9	25.3	51.1	17.1	34.5	36.5
11	a-C17	2.3	3.0	7.5	3.5	12.3	11.7
12	n-C17	1.5	4.3	1.7	1.6	1.4	2.2
13	u.i.[c]	0.3					
14	i-C18	0.5	0.7	1.2	1.2	2.0	1.3
15	n-C18	0.9	1.6	1.3	0.7	1.3	3.8
16	n-C18:1	0.9	1.3	0.3	0.4	2.4	0
17	i-C19	0.3	0.5	0.3	0.2	0	0
18	u.i.		0.3				
19	u.i.	0.4	0.4	0.6			
20	i-C20	0.2	0.2	0.3	0.3		

[a] All data are given as percentage of peak area of total fatty acid fraction. Extracts and fatty acid methyl esters were prepared according to either the method of Kates et al. (7) or that of Dunlap and Perry (2). Since this latter method gave the same qualitative but better quantitative results, it was applied throughout most of the experiments. Samples of 0.5 to 2.0 μliters were run on a Pye series 104 gas chromatograph, on a 5-ft glass column with 10% diethyleneglycol succinate on 100 to 120 mesh Diatomite C at 170 C. The gas flow rates were 40 ml/min each for N_2 and H_2, and 700 ml/min for air. The method of James (5) was followed for bromination of the samples. Fatty acid methyl esters were identified by comparing their retention times with those of commercially available standards: quantitative mixture H108, BC Mix-L no. 19189, and B Mix-I no. 19190, all from Applied Science Laboratories, Inc.

[b] Four to six very small peaks, representing less than 0.5% of the total fatty acid fraction appear before the i-C13 peak.

[c] u.i., unidentified.

pair, the decrease of i-C16 fatty acids, and the more than doubling of the a-C17 plus i-C17 fatty acid pair (Table 2, g, h, and k). The total amount of C16 compounds shows only slight decrease with increasing temperature (Table 2, l). However, the individual straight and iso-C16 compounds are both quite sensitive to temperature changes (Table 2, h and i). The decrease of the iso-form corresponds to an increase of n-C16 fatty acids. The decrease of i-C16 fatty acid is, however, not entirely compensated by the increase of n-C16 fatty acid, as reflected by the n-C16 to i-C16 ratio. The compensation lies in the formation of the longer-chain branched C17 fatty acids. Surprisingly, the anteiso-compounds, especially a-C17 compounds with their much lower melting point than the corresponding straight and iso-forms (6), were found to increase with temperature.

Though the decrease of i-C15 fatty acids and the increase of i-C17 fatty acids are the predominant events in both strains, the changes in the amounts of these compounds in *B. caldotenax* are smaller than in *B. caldolyticus* (Table 1, peaks 5 and 10). The amount of the n-C16 component almost doubles in both strains when the temperature is raised from 60 to 80 C, but *B. caldotenax* contains twice the amount of this compound, possibly compensating for the smaller i-C17 increase.

Furthermore, the shift from br.-C15 to br.-C17 as the predominant compounds occurs in *B. caldolyticus* at 80 C, whereas this happens at 70 C in *B. caldotenax*. In general, the data reflect a more intense change in *B. caldolyticus* when the temperature is raised to 10 C above the optimal temperature than is found by lowering the temperature to 60 C, i.e., 10 C below the temperature optimun. In *B. caldo-*

TABLE 2. *Groups and pairs of fatty acids from Bacillus caldolyticus and Bacillus caldotenax at 60, 70, and 80 C*[a]

Fatty acid group	*B. caldolyticus*			*B. caldotenax*		
	60 C	70 C	80 C	60 C	70 C	80 C
a. Total branched	82.8	72.0	78.8	81.0	78.1	65.3
b. Total straight	15.5	26.9	20.1	19.2	21.9	34.7
c. Branched, odd (15+17)	58.5	58.1	68.3	51.1	59.1	55.1
d. Branched, even (14+16)	23.0	12.2	8.2	28.2	17.0	8.9
e. Straight, odd (15+17)	5.4	10.2	2.7	3.8	2.3	3.4
f. Straight, even (14+16)	8.1	14.0	14.6	14.3	17.6	25.0
g. a-C15 + i-C15	38.3	29.8	9.9	30.5	12.3	6.9
h. i-C16	20.0	11.8	7.9	27.2	16.6	8.5
i. n-C16	6.6	12.7	13.7	13.3	16.8	24.0
k. a-C17 + i-C17	20.2	28.3	58.6	20.6	46.8	48.2
l. n-C16 + i-C16	26.6	24.5	21.6	40.5	33.4	32.5
m. n-C16/i-C16	0.33	1.07	1.73	0.49	1.01	2.82

[a] Data shown are percentages of total fatty acids, except for line m, which is the ratio of n-C16 fatty acids to i-C16 fatty acids.

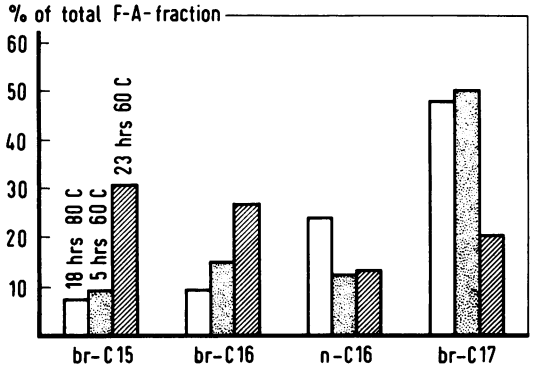

FIG. 1. *Alterations of fatty acid ratios of Bacillus caldotenax with temperature and time. Both strains were grown under optimal conditions (3), at either 60, 70, or 80 C. Cells were harvested in the late log or early stationary phase. Preliminary experiments had shown that the fatty acid composition does not change with time, if cultures are inoculated at a given temperature from batches of the same temperature. Even cells from the late stationary phase showed only negligible alterations.*

tenax, on the other hand, the changes are relatively minor when the cells are grown at 10 C below their optimum, but much more apparent upon another 10 C drop to 60 C, i.e., 20 C below the optimum.

Another factor is introduced if the cells are not grown at a given temperature for the time necessary to reach the late log phase but if cells from an 80 C batch are cultivated at 60 C for a shorter or longer period of time. This became evident when *B. caldotenax* was grown at 60 C either for only 5 hr, or for 23 hr. A comparison of these data show that some of the changes in the relative abundance of the predominant fatty acids occur very fast, while others lag behind (Fig. 1). The amount of br.-C15 fatty acid changes only slightly after 5 hr at 60 C, while almost one-third of the total change of br.-C16 fatty acid has already occurred at that time. The decrease of n-C16 fatty acids is almost completed after 5 hr at 60 C, whereas hardly any change is observed with i-C17 fatty acids at that time. In general, this shows that it takes more time for the branched, odd-numbered fatty acids to either increase or decrease than for the straight and iso-C16 fatty acids, which both react very fast to the temperature change. Compared to the other fluctuations, this again emphasizes the high sensitivity of the n-C16 plus i-C16 pair.

LITERATURE CITED

1. Daron, H. H. 1970. Fatty acid composition of lipid extracts of a thermophilic *Bacillus* species. J. Bacteriol. **101**:145–151.
2. Dunlap, K. R., and J. J. Perry. 1967. Effect of substrate on the fatty acid composition of hydrocarbon-utilizing microorganisms. J. Bacteriol. **94**:1919–1923.
3. Heinen, W. 1971. Growth conditions and temperature-dependent substrate specificity of two extremely thermophilic bacteria. Arch. Mikrobiol. **76**:2–17.

4 Heinen, W., H. P. Klein, and C. M. Volkmann. 1970. Fatty acid composition of *Thermus aquaticus* at different growth temperatures. Arch. Mikrobiol. **72**:199–202.
5. James, A. T. 1960. Qualitative and quantitative determination of the fatty acids by gas-liquid chromatography. Methods Biochem. Anal. **8**:1–59.
6. Kaneda, T. 1963. Biosynthesis of branched-chain fatty acids. I. Isolation and identification of fatty acids from *Bacillus subtilis* (ATTCC 7059). J. Biol. Chem. **238**: 1222–1228.
7. Kates, M. B. Palameta, C. N. Joon, D. J. Kushner, and N. E. Gibbons. 1966. Aliphatic diether analogs of glyceride derived lipids. IV. The occurence of di-O-dihydrophytyl-glycerol ether containing lipids in extremely halophilic bacteria. Biochemistry **5**:4092–4099.
8. Shen, P. Y., E. Coles, J. L. Foote, and J. Stenesh. 1970. Fatty acid distribution in mesophilic and thermophilic strains of the the genus *Bacillus*. J. Bacteriol. **103**:479–481.