JOURNAL OF BACTERIOLOGY, Jan. 1974, p. 212-214

Vol. 117, No. 1
Printed in U.S.A.

Effects of pH and Temperature on the Fatty Acid Composition of *Bacillus acidocaldarius*

M. DE ROSA, A. GAMBACORTA, AND J. D. BU'LOCK

CNR Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico, Arco Felice, Napoli, Italy, and Microbial Chemistry Laboratory, Department of Chemistry, The University, Manchester, England

Received by publication 10 January 1973

The fatty acid composition of lipid extracts from cells of *Bacillus acidocaldarius* grown at temperatures of 50 to 70 C and pH values of 2 to 5 was determined by gas chromatography of the methyl esters. The most abundant fatty acids are 11-cyclohexylundecanoic and 13-cyclohexyltridecanoic, followed by anteiso- and iso-heptadecanoic; unsaturated acids are absent. Highly aerated cultures produce more of the iso and anteiso acids and less of the cyclohexyl acids. The effects of temperature and pH are interdependent; at lower pH, increasing temperature raises the proportion of the iso and anteiso acids, but at higher pH the effect of increasing temperature is reversed and the proportion of the cyclohexyl acids is increased.

Bacillus acidocaldarius is characterized by tolerance of high temperature and acidity (50 to 70 C, pH 2 to 5) (1) and by its unusual lipids containing high proportions of 11-cyclohexylundecanoic and 13-cyclohexyltridecanoic acids (3). We considered that a study of the effects of culture conditions upon the fatty acid composition of this unusual organism might provide useful comparative data.

MATERIALS AND METHODS

Growth conditions for the Agnano strain of *B. acidocaldarius* are fully described (4; De Rosa, Gambacorta, and Bu'Lock, G. Microbiol., in press). Cells were grown with aeration on a medium containing 0.1% glucose, 0.1% yeast extract, and mineral salts; aeration was normally at 0.5 liters per liter of culture per min, and pH was adjusted with H_2SO_4. The pH does not change significantly during growth of the culture. Normally, at the end of the growth phase, cells were centrifuged, washed with 0.1 M NaCl, lyophilized, and extracted (Soxhlett) with 1:1 $CHCl_3$-CH_3OH; the crude lipid extract was saponified with 10% KOH-CH_3OH (reflux, 6 h) and esterified with diazomethane. After gas chromatography (80 m steel capillary coated with diethyleneglycol succinate at 220 C with 1 ml of pure N_2 per min at 2.2 kg/cm^2), the component acids were identified from plots of the logarithm of retention time versus chain length and calibrated with standards for the cyclohexyl acids (3), and amounts were estimated from peak areas measured graphically. The complete absence of unsaturated esters inferred from gas chromatography was confirmed by thin-layer chromatography on $AgNO_3$-treated silica gel.

RESULTS AND DISCUSSION

Measurements made over the range of temperature and pH which *B. acidocaldarius* tolerated are summarized in Table 1. Some other variables were investigated at the optimal temperature and pH (60 C, pH 3.0; specific growth rate, 0.58 generations per h). Cells early in the growth phase contained more of the anteiso-C_{17} acid (at 23 h, 13% iso-C_{17} and 20% anteiso-C_{17}; at 90 h, 20 and 8%, respectively); other components varied less. Aeration had a marked effect: increasing the sparging rate from 0.16 to 1.60 liters per liter per min lowered the total of cyclohexyl acids from 66 to 38%, simultaneously increasing the content of iso-C_{17} and anteiso-C_{17} acids from 29 to 53%.

In Table 1, the acids are grouped biogenetically, according to Kaneda (6). For comparison with other bacteria, it is useful to consider the composition of the mixture of acyclic acids only, excluding the unique cyclohexyl acids. Thus at 60 C, pH 3.0, the acyclic mixture comprises 8% n-C_{2n}, 2% n-C_{17}, 15% iso-C_{16}, 39% iso-C_{2n+1}, and 34% anteiso-C_{2n+1}, and the average chain length is 15.9. Such data are well within the range for typical mesophilic *Bacillus* species (6, 7). However, within this spectrum of acyclic acids, the effects of both temperature and pH are considerable. At pH 3.0 the effect of raising the temperature between 50 and 70 C is to increase the proportion of iso-C_{2n+1} acids, apparently at the expense of the n-C_{2n} and iso-C_{2n}, but at pH 5.0 with the same temperature variation the result is more complex: the iso-C_{2n+1} acids rise, and the iso-C_{2n} fall, as before, but the anteiso-C_{2n+1} acids fall to a minimum (at 55 C) and then rise again while the n-C_{2n} acids rise to a maximum (at 60 C) and then fall. Systematic effects of changing pH at a given temperature

are harder to determine.

This complexity reflects a metabolic situation which is by no means simple; the biogenetic groups arise from different acyl-coenzyme A (CoA) species competing as "starters" for fatty acid synthetase, and whereas the starters for the iso- and anteiso-acids are formed either by breakdown of the branched-chain amino acids or in competition with their synthesis, the n-C_{2n} and n-C_{2n+1} acids derive from acetyl- and propionyl-CoA whose levels are less closely linked to amino acid metabolism. A priori arguments concerning the effects of temperature on the balance between these systems are lacking. However, at all pH values the temperature effects observed here are quite different from those in *B. stearothermophilus*, which greatly increases its content of n-C_{2n} acids at higher temperatures (2, 9).

The cyclohexyl acids are best considered separately since there are no comparable data for other bacteria. Biogenetically they are quite remote from the acyclic acids, since they arise from a cyclohexyl-carboxylic acid starter formed reductively from shikimic acid (4), and in Table 1 it is the proportion of cyclic to acyclic acids which undergoes the greatest changes with varying temperature and pH. The effect of aeration on this ratio, already noted, is entirely consistent with the reductive nature of the synthesis of the cyclic starter, but the interdependent effects of temperature and pH are less immediately intelligible. Whereas at pH 5.0 increasing temperature raises the proportion of cyclic acids (from 27% at 50 C to 69% at 70 C), at pH 3.0 the proportion falls, from 65% at 50 C to 49% at 70 C. The variation at a given temperature with varying pH is equally complex, and at least two quite distinct mechanisms must be operating simultaneously to explain the observed pattern.

Tentatively, we presume that both energy demands and oxygen availability influence the pattern of carbon utilization in this aerobic sugar-utilizing organism. The observations cover a thousandfold range in external hydrogen ion concentration (the internal pH of the cells is near neutrality) while the solubility of air-oxygen in water at 70 C is about two-thirds of that at 50 C. We suggest that, at low pH, ion-pumping mechanisms impose a considerable energy demand, and that a pattern of metabolism attuned to this demand is also favorable for the production of the shikimate-derived acids. At higher pH this particular demand will be much less stringent, but under these conditions the effect of increasing temperature on oxygen availability may be overriding, with a pattern of carbon metabolism favoring oxidative synthesis of the acyclic starters at 50 C and reductive synthesis of the shikimate-derived acids at 70 C. Unfortunately, oxygen electrodes capable of satisfactory operation over the required range of temperature and pH were not available.

In describing the only other source of 11-cyclohexylundecanoic acid, a minor component (3%) in the fatty acids from rumen bacteria, Hansen (5) noted that it was possible to confound such an unexpected component with more common acids (e.g., with methyl stearate on an Apiezon L column or with methyl linoleate on poly ([ethylene glycol adipate]); hence a

TABLE 1. *Fatty acid composition (%) of lipids from B. acidocaldarius grown at different temperature and pH*

Fatty acid	pH 2.0			pH 3.0					pH 4.0					pH 5.0				
	50[a]	55	60	50	55	60	65	70	50	55	60	65	70	50	55	60	65	70
n-C_{2n} C_{14}	+[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
n-C_{2n} C_{16}	5	5	3	5	5	3	3	1	6	5	4	3	2	6	7	6	3	2
n-C_{2n} C_{18}	+	+	+	1	+	+	+	+	1	1	+	+	+	+	1	1	1	+
n-C_{17}	1	1	+	2	1	1	+	+	3	1	1	1	1	2	1	1	1	+
Iso-C_{16}	5	5	3	6	7	6	7	2	14	12	3	6	3	18	13	7	7	2
Iso-C_{2n+1} C_{15}	1	2	2	1	2	1	3	2	3	2	2	3	4	3	5	3	2	2
Iso-C_{2n+1} C_{17}	6	14	15	10	16	15	22	28	16	15	14	21	24	15	16	15	16	10
anteiso-C_{2n+1} C_{15}	1	1	1	1	+	1	+	1	3	2	1	1	2	4	2	2	1	1
anteiso-C_{2n+1} C_{17}	16	16	18	10	11	13	13	16	18	18	18	17	21	26	14	14	14	14
cyclo-C_{2n+1} C_{17}	27	27	28	27	28	28	24	21	19	26	25	26	23	14	28	30	30	34
cyclo-C_{2n+1} C_{19}	38	30	31	38	30	31	28	28	19	19	27	22	22	13	15	22	25	35

[a] Temperature (C).

[b] Fatty acid composition (%) of lipids.

single system may not be sufficiently discriminatory. In *B. acidocaldarius* the acids are major components and analysis is simplified by the absence of unsaturated acids, but the comment remains valid, and until the *cyclo*-C_{2n+1} acids have been specifically sought in other bacteria it is impossible to judge whether their occurrence will allow us to locate any taxonomic relatives of *B. acidocaldarius;* this is particularly important because of the possible geochemical significance of the lipid mixture in *B. acidocaldarius* (4).

LITERATURE CITED

1. Darland, G., and T. D. Brock. 1971. *Bacillus acidocaldarius*, and acidophilic thermophilic spore-forming bacterium. J. Gen. Microbiol. **67**:9-15.
2. Daron, H. H. 1970. Fatty acid composition of lipid extracts of a thermophilic *Bacillus* species. J. Bacteriol. **101**:145-151.
3. De Rosa, M., A. Gambacorta, L. Minale, and J. D. Bu'Lock. 1971. Cyclohexane fatty acids from a thermophilic bacterium. Chem. Commun. p. 619-620.
4. De Rosa, M., A. Gambacorta, L. Minale, and J. D. Bu'Lock. 1972. A new biosynthetic pathway: the formation of ω-cyclohexyl fatty acids from shikimate in an acidophilic thermophilic bacillus. Biochem. J. **128**:751-754.
5. Hansen, R. P. 1967. 11-Cyclohexylundecanoic acid: its occurrence in bovine rumen bacteria. Chem. Ind. p. 1640-1641.
6. Kaneda, T. 1967. Fatty acids in the genus *Bacillus*. J. Bacteriol. **93**:894-903.
7. Shen, P. Y., E. Coles, J. L. Foote, and J. Stenesh. 1970. Fatty acid distribution in mesophilic and thermophilic strains of the genus *Bacillus*. J. Bacteriol. **103**:479-481.
8. Yao, M., H. W. Walker, and D. A. Lillard. 1970. Fatty acids from vegetative cells and spores of *Bacillus stearothermophilus*. J. Bacteriol. **102**:877-878.