MAJOR OCCURRENCE OF CIS-Δ^5 FATTY ACIDS IN THREE PSYCHROPHILIC SPECIES OF BACILLUS*

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Monounsaturated fatty acids occur in Bacillus insolitus, Bacillus psychrophilus, and Bacillus globisporus in unusually large proportions for this genus. These unsaturated fatty acids have been identified as exclusively the cis-Δ^5 isomers. Large proportions of branched-chain and small proportions of straight-chain saturated fatty acids common to this genus have also been found.

Introduction

Stokes and his associates have isolated (1) and identified (2) several species of psychrophilic bacilli capable of growing at temperatures as low as -2 C. Organisms such as these bacilli must have some distinctive features specifically providing for low temperature growth. One obvious possibility is that they may produce lipids of unusually low softening temperature. To examine this idea, the fatty acids occurring in these organisms have been investigated.

Members of the genus Bacillus have a characteristic physiological nature: their fatty acids are largely methyl branched acids (iso and anteiso series) whereas normal fatty acids, namely, myristic, palmitic, and stearic, occur as minor fatty acid components. Furthermore, unsaturated fatty acids in these organisms are generally insignificant or in very small proportions (3,4). Two species, both pathogenic, were previously shown to contain significant proportions of cis-Δ^{10}-unsaturated fatty acids (5,6). I have now found that psychrophilic species of Bacillus, B. insolitus, B. psychrophilus, and B. globisporus also contain important amounts of unsaturated fatty acids.

Experimental and Discussion

Microorganism: B. insolitus, B. psychrophilus, and B. globisporus, kindly supplied by Dr. J. L. Stokes, were maintained on Tripticase Soy Broth (BBL) in the cold.

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Preparation of Bacterial Fatty Acids: Inocula for large preparations of cells were obtained by growing the organisms for 16-24 hrs in 100 ml of Tripticase Soy Broth at 22 C in 500 ml Erlenmeyer flasks on a rotary shaker. One liter of Tripticase Soy Broth in a 2.8 l. Fernbach flask was then inoculated with 10 ml of the culture and incubated for 16 hrs under similar conditions. Growth was measured with a Klett-Summerson colorimeter with a no. 66 filter and ranged from 220 to 234 Klett units. The cells were centrifuged and washed once with 0.85% NaCl. An average of 2 to 3 g of wet, packed cells were recovered from each of six runs. The fatty acids were isolated and methylated with diazomethane by methods previously published (7, 8).

Identification of Bacterial Fatty Acids: When a small portion of the extracted, methylated fatty acid sample was subjected to thin layer chromatography on Silica Gel G (Brinkman Instruments Inc.) using 10% ethyl ether in n-hexane as developer, only one spot could be detected with Rhodamine 6G spray (0.002% in water). Its Rf, 0.47, was identical to that of methyl palmitate. When the chromatography was conducted on Silica Gel G impregnated with $AgNO_3$, two spots were obtained. The Rf of the upper, 0.45, was identical to that of methyl palmitate and the Rf of the lower, 0.25, was the same as that of methyl palmitoleate. Hence it was concluded that the fatty acids in the sample consisted of two classes only, alkanoic and monounsaturated fatty acids, with no contribution from acids with polar functional groups or from polyunsaturated acids.

The major portion of the methylated fatty acid sample was separated into the two classes by column chromatography on $AgNO_3$ impregnated silicic acid (9). The saturated fraction accounted for 68, 82, and 74% of the total in the cases of B. insolitus, B. psychrophilus and B. globisporus, respectively.

The components of the saturated fraction were characterized by gas chromatography on a 100 ft ethylene glycol adipate support-coated open tubular column (EGA-SCOT)(Perkin-Elmer Corp.), and by mass spectroscopy of brominated, hydrogenated, deuterated, and untreated portions of the fraction. They have been identified as iso-C_{13}, anteiso-C_{13}, iso-C_{14}, n-C_{14}, iso-C_{15}, anteiso-C_{15}, n-C_{15}, iso-C_{16}, n-C_{16}, iso-C_{17}, anteiso-C_{17}, iso-C_{18}, and n-C_{18}. Anteiso-C_{15} was the major component in all the three organisms. A detailed account of the identification sequence will be reported elsewhere.

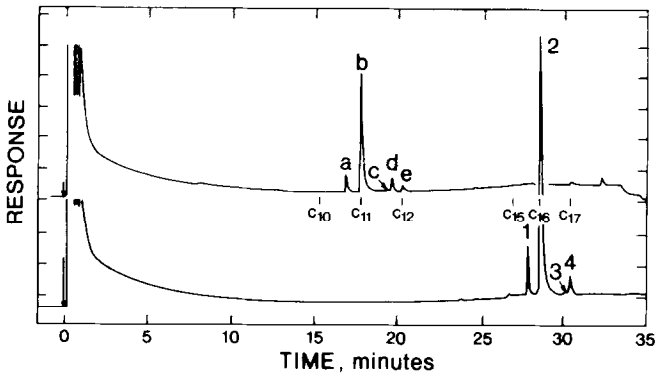

Fig. 1. Gas-liquid chromatograms of the unsaturated fraction of the fatty acids isolated from Bacillus insolitus (W16B). A 100-ft support-coated column with ethylene glycol adipate polymer was operated isothermally for 2 min at 170° and then the column temperature was programmed at a rate of 2° per min up to a final temperature of 210°. The upper and lower figures are the chromatograms of the unsaturated fraction after, and before treatment with permanganate-periodate mixture, respectively. Positions of normal fatty acid standards are indicated in the middle.

The identification of the components of the unsaturated fraction is presented here. The bottom half of Fig. 1 shows the gas-liquid chromatogram of the unsaturated fraction from B. insolitus. On the EGA column, peaks 1, 2, 3, and 4 show equivalent chain lengths (10) 0.05 carbons longer than those of iso-C_{16}, n-C_{16}, iso-C_{17}, and anteiso-C_{17} respectively. After the components were hydrogenated, equivalent chain lengths were identical to the corresponding saturated acids listed above.

Mass numbers and carbon skeletons of the components were determined by mass spectroscopy on a Perkin-Elmer model 270 GC-DF analytical mass spectrometer. The methyl esters of material corresponding to peaks 1, 2, 3, and 4 gave the parent mass numbers (P) of 268, 268, 282, and 282 respectively. When any of the components was hydrogenated or deuterated, the mass number correspondingly increased by 2 or 4.

After catalytic hydrogenation, the component corresponding to peak 2 gave a fragmentation pattern typical of a normal fatty acid showing P-29, P-31, P-43, 143, 87, 74, and others in intensities identical to those of methyl palmitate. Reduced material from peaks 1 and 3 gave patterns similar to those of methyl palmitate and methyl magarate respectively but, as expected for iso fatty acids, a characteristic

fragment corresponding to P-65 and a higher intensity of P-43 were observed (11). The reduced peak 4 component gave the fragments of P-61 and P-79 in nearly equal intensities characteristic of the anteiso series which are not observed with normal or iso fatty acids (11). Using fatty acids from Bacillus subtilis previously identified in this laboratory (7) as reference compounds, mass spectra of hydrogenated material corresponding to peaks 1, 2, 3, and 4 were found to be identical to those of iso-C_{16}, n-C_{16}, iso-C_{17}, and anteiso-C_{17} fatty acids, respectively.

The double bond was located by permanganate-periodate oxidation (12) followed by gas chromatography of the oxidation products on two columns, the EGA-SCOT, and an SE-30 (9%)-ethylene glycol adipate (1%) coated on Anakrom ABS (Analab Inc.). As can be seen from the peak heights in the top half of Fig. 1, the relative proportions of peaks a, b, c, and d are almost identical to those of peaks 1, 2, 3, and 4 in the chromatogram of the original material. Peaks a, b, c, and d were identified as iso-C_{11}, n-C_{11}, iso-C_{12}, and anteiso-C_{12} fatty acids; that is, they have structures like the respective original acids except that they are five carbons shorter. Therefore, the original fatty acids must be the Δ^5 isomers. An additional small peak, e, was detected in the oxidized sample. This was identified as n-C_{12} fatty acid on the two columns. Hence Δ^5-n-C_{16} fatty acid, peak 2, may contain a very small amount of the Δ^3 isomer.

In general, the unsaturated fatty acids occurring in biological systems are usually cis isomers. Since the IR spectrum of the unsaturated fraction showed none of the absorption at 10.3 μ characteristic of trans isomers (13), it was concluded that the unsaturated fatty acids from B. insolitus are also cis isomers.

In summary, the unsaturated fatty acids isolated from B. insolitus are identified as the cis-Δ^5 isomers of iso-C_{16}, n-C_{16}, iso-C_{17} and anteiso-C_{17} fatty acids. Cis-5-hexadecenoic acid is the major component, accounting for 20% of the total fatty acids.

Similarly, the same four fatty acids are identified as the major components of the unsaturated fractions from B. psychrophilus and B. globisporus although the relative ratios of the fatty acids are significantly different. Cis-Δ^5-anteiso-C_{17} acid is,

in both organisms, the most abundant, accounting for 10 and 15% of the total, respectively.

Members of Bacillus generally do not synthesize unsaturated fatty acids in any significant amount. In this respect, the three species studied in this investigation deviate from others in this genus. It is likely that the occurrence of unsaturated fatty acids in these organisms is related to their psychrophilic growth. The function of the unsaturated fatty acids, however, may not be only lowering the softening temperature of the lipids. If so, Δ^9-isomers (which are found commonly in nature) would satisfy this function as well as, or perhaps better than do Δ^5-isomers since melting points of Δ^9-isomers are generally similar to, or even lower than, those of the corresponding Δ^5-isomers (14).

Thus the significance of the unusual position of the double bond is not clear. This mystery is similar to that associated with the location of unsaturation in the Δ^{10}-position in the previously studied pathogens (5).

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