JOURNAL OF CLINICAL MICROBIOLOGY, July 1980, p. 127-130
0095-1137/80/07-0127/04$02.00/0

Vol. 12, No. 1

Gas-Liquid Chromatography of Bacterial Fatty Acids with a Fused-Silica Capillary Column

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The use of a flexible, fused-silica capillary column for gas-liquid chromatographic analysis of bacterial fatty acids is illustrated with *Propionibacterium acnes*, *Propionibacterium shermanii*, and a standard methyl ester mixture.

Determination of bacterial fatty acid composition by gas-liquid chromatography (GLC) has proved to be a valuable additional means for rapid identification of a variety of bacteria (2, 4, 6, 10). Recent examples in this laboratory are *Legionella pneumophila* and some *Legionella*-like organisms (1, 5, 9). In general, the GLC analysis of fatty acids (as methyl ester) has been done on glass columns of varying lengths, with internal diameters generally from 2 to 4 mm, packed with polar or nonpolar stationary-phase materials (4). Not all of the acids in a complex bacterial mixture, however, are separated on packed columns, and some appear as shoulders on the leading or trailing edge of other peaks in the GLC chromatogram (4). Increased separa-

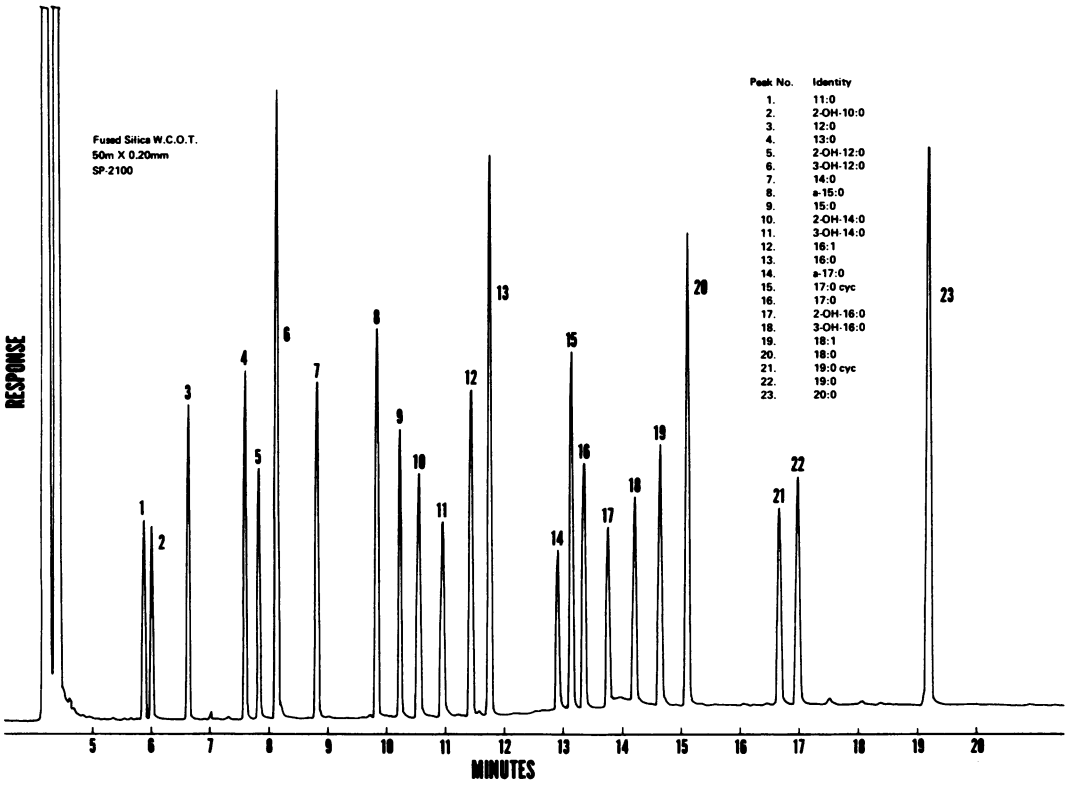

FIG. 1. *Gas chromatogram of a fatty acid methyl ester standard analyzed on a fused-silica capillary column (50 m by 0.20 mm). Helium flow, 1.0 ml/min. Peak designation: number before the colon refers to the number of carbon atoms and the number after it refers to the number of double bonds. i, Branched-chain acid with the methyl group at the penultimate carbon atom; a, branched-chain acid with the methyl group at the antipenultimate carbon; 2- or 3-OH refers to a hydroxyl group at the 2 or 3 carbon atom; cyc, a cyclopropane acid.*

tion of poorly resolved components should be possible with a capillary GLC column such as the flexible, fused-silica glass capillary column, which was recently introduced by several commercial companies.

A fused-silica capillary column (50 m by 0.2

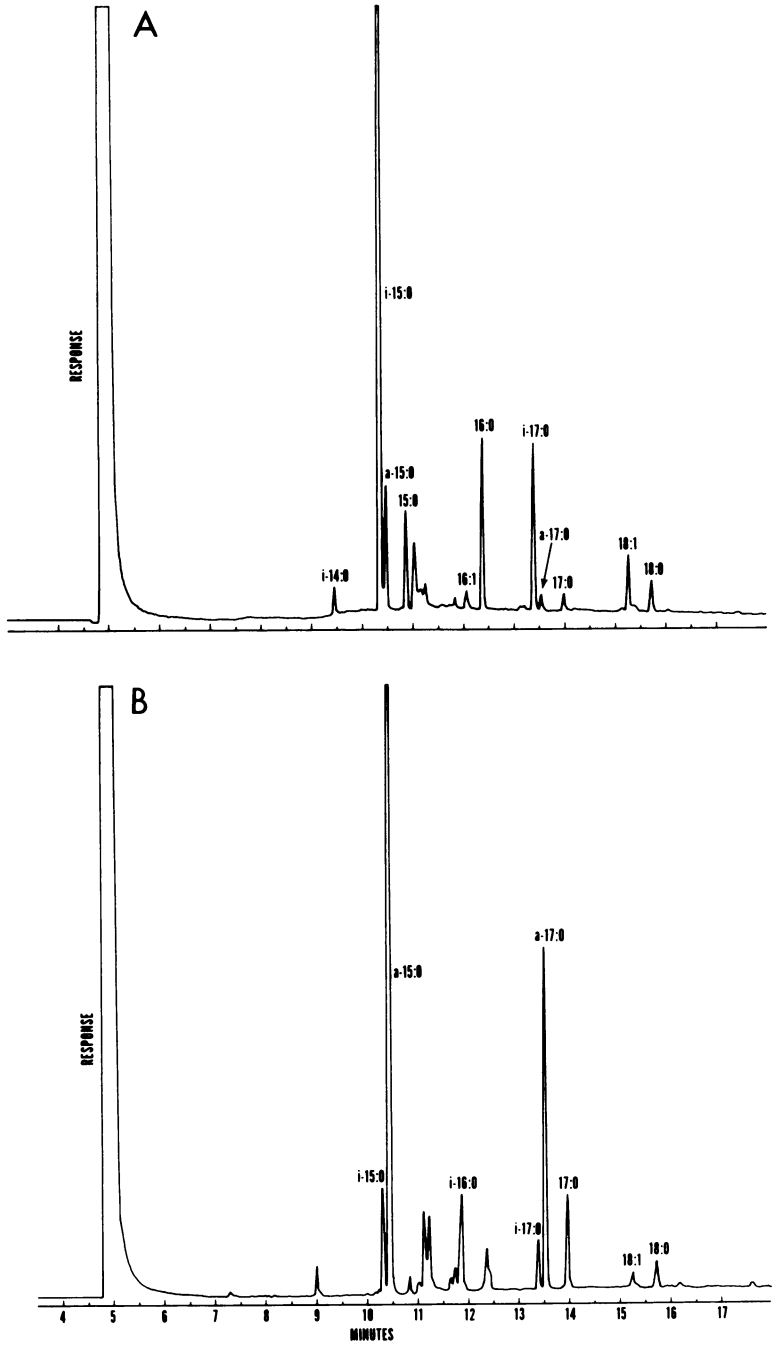

FIG. 2. *Gas chromatogram of esterified fatty acids from saponified whole cells of P. acnes (A) and P. shermanii (B) analyzed on a fused-silica capillary column (50 m by 0.20 mm). Helium flow = 0.8 ml/min. See Fig. 1 for peak designation.*

mm, inside diameter) with SP-2100 as stationary phase was purchased from Hewlett-Packard, Avondale, Pa., and installed in a Varian model 3700 gas chromatograph equipped with an all-glass capillary system (Varian Instruments, Palo Alto, Calif.). The column was conditioned for 2 days at temperatures from 120°C to a maximum of 260°C and then connected to a glass-lined flame ionization detector. The injection temperature was maintained at 250°C, and the detector temperature was maintained at 300°C. Helium was used as carrier gas at a flow rate of 0.8 or 1.0 ml/min. The sample size was 0.5 μl, which was split 50:1 to give 0.01 μl of sample on the column. For analysis of samples, the column temperature was set at 195°C for 5 min and then temperature-programmed at 7°C/min to a final temperature of 250°C.

Shown in Fig. 1 is a chromatogram of a standard mixture of fatty acid methyl esters run on the capillary column. It is clear that base-line resolution was obtained for each of the 23 components of this complex mixture. This degree of separation of methyl esters has not been attained with a packed column under any conditions of chromatography. For example, with a packed column, the 17-carbon acid series (Fig. 1, peaks 14, 15, and 16) is either not resolved or appears as one peak with leading or tailing shoulders (4). Moreover, hydroxy acids often tail on a packed column, but on the capillary column these acids (Fig. 1, peaks 2, 5, 6, 10, 11, 17, and 18) appear as sharp, symmetrical, well-resolved peaks. Additional studies, however, are required to determine whether the hydroxy acids will also tail on the capillary column after extended column use.

An additional significant characteristic of the capillary column is the capacity to resolve positional isomers of acids with the same carbon chain length. Many bacterial species are known to contain branched-chain fatty acids which generally are of two types: the iso form, in which the methyl group is located on the penultimate carbon, and the anteiso form, in which the methyl group is located on the antipenultimate carbon atom. On a packed column (with nonpolar stationary phase), these isomers co-elute and thus appear as one peak in the chromatogram; however, on the capillary column the iso and anteiso isomers are essentially completely resolved. This is illustrated in Fig. 2, which shows chromatograms of the cellular fatty acids of two bacterial species known to contain major amounts of branched-chain acids (7, 8). The top chromatogram shows that the most abundant acid in *Propionibacterium acnes* (3) is an iso-branched-chain 15-carbon acid (i-15:0) which elutes from the column at approximately 10.4 min. The peak immediately after i-15:0 at a retention time of approximately 10.5 min is the anteiso 15-carbon acid (a-15:0). It is apparent that these isomers as well as the iso and anteiso 17-carbon isomers (retention time: approximately 13.4 and 13.5 min, respectively) are essentially completely resolved. The bottom chromatogram shows that the major acids in *Propionibacterium shermanii* are the a-15:0 and a-17:0 acids, with only small amounts of i-15:0 and i-17:0 which are the major acids in *P. acnes*. These data confirm earlier results which indicated that these species can be distinguished by cellular fatty acid composition (7, 8). As illustrated in Fig. 2, these differences in cellular fatty acids can be measured rapidly and accurately with a GLC capillary column.

Increased resolution and separating efficiency of glass capillary columns compared with packed columns are well known (4). Their limited use can be attributed to high costs and extreme fragility. The problem of fragility is essentially eliminated with the new fused-silica column, since it is flexible enough to be virtually unbreakable in normal usage. The relatively high costs (approximately $300.00) may be justified if future studies establish that the column retains its inertness and high efficiency after extended use.

We thank G. L. Lombard, Anaerobe Section, Center for Disease Control, for growing the *Propionibacterium* cultures.

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