**Gas chromatographic procedure for identification of bacterial metabolic products.**

Strains were cultured on peptone/yeast extract/glucose (PYG) agar plates under anaerobic conditions for 1-5 d in anaerobe jars containing 80% $N_2$, 10% $H_2$ and 10% $CO_2$.

**Chromatographic conditions.** End products produced in the medium were assayed by GC using the method described in the *Anaerobe Laboratory Manual* (Holdeman *et al.*, 1977). The GC was equipped with a 15 meter x 0.53 mm Nukol fused silica column (Supelco, Inc.) and a flame ionization detector. For analysis of the samples, the column temperature was maintained at 75°C for 2 min and then temperature programmed to 195°C at 15°C per minute. Metabolites were identified after injecting 1 µl sample in the GC in the splitless mode and comparing with chromatograms of the standards.

**Standard solutions.** For volatile fatty acids (VFAs), a standard solution containing a 1:5000 dilution each of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic and heptanoic acids in tert-Butyl methyl ether was prepared. Additionally, an internal standard solution was prepared by dissolving 200 µl heptanoic acid in 100 ml deionised water and neutralising with 5 M NaOH. For non-volatile fatty acids (nVFAs) a standard solution containing pyruvic, lactic (10 mmol), oxalacetic, oxalic, methylmalonic, malonic, fumaric, succinic (5 mmol) and phenylacetic (1,2 mmol) acids was prepared, and 500 µl was pretreated as the culture samples.

It should be noted that in cultures that produce very large amounts of propionic acid, traces of the free (unmethylated) acid may interfere with the fumaric acid peak. Also, when interpreting nVFA chromatograms, corrections must be made for amounts of acids in the unoculated media.

**Sample pretreatment.** VFA: 8 agar discs (9 mm diam.) were punched out of the culture and transferred to a centrifuge tube containing 0.5 ml 10% $H_2SO_4$ and 100 µl internal standard solution. After capping, the tube was placed in a heat block at 100 ºC until the agar was melted. The cooled sample was extracted with 1.5 ml tert-Butyl methyl ether using a Vortex mixer and then centrifuged. A portion of the upper layer was transferred to an injection vial and capped.

nVFA: 4 agar discs were punched out of the culture and transferred to a centrifuge tube containing 1 ml methanol and 0.5 ml $H_2SO_4$. After capping, the tube was placed in a heat block at 100 ºC until the agar was melted, and then in a heat block at 70 ºC for 30 min to obtain methyl derivatives of non volatile fatty acids. The cooled sample was extracted with 1 ml chloroform using a Vortex mixer and then centrifuged. A portion of the lower layer was transferred to an injection vial and capped.

References

Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).
1977. Anaerobe Laboratory manual, 4th ed. Virginia
Polytechnic Institute and State University, Blacksburg.