Analysis by gas liquid chromatography of production of volatile fatty acids by anaerobic bacteria grown on solid medium

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SUMMARY Volatile fatty acids produced in Robertson's cooked meat medium by a range of clinically relevant anaerobes were compared by gas liquid chromatography with those produced in blood agar. The same volatile fatty acid profiles were obtained in both media, although the concentration of acids was lower in blood agar. We conclude that detection of volatile fatty acids from a pure culture of an organism on solid medium is practicable and offers advantages over the conventional technique.

The value of gas liquid chromatography in identifying anaerobic bacteria by detecting the short chain fatty acids which they produce has been clearly established.^{1,2} A profile of the volatile fatty acids produced is readily obtained and is a useful adjunct to biochemical tests in the formal identification of anaerobic isolates.

Conventionally, a pure culture of the organism is first obtained on solid medium and subcultured into a liquid medium containing one or more carbohydrates. The liquid medium is then incubated for 48 hours and an extract analysed by gas liquid chromatography. If the same short chain fatty acids are present in similar amounts in extracts prepared from the initial pure culture of the organism on solid medium this would obviate the need to incubate broth cultures, consequently saving time and culture medium. A method has recently been described in which production of volatile fatty acid by *Clostridium difficile* was compared in primary isolation medium and in cooked meat broth.³ The same volatile fatty acids were found in similar quantities in both media.

We used a similar method to examine the production of volatile fatty acids by a range of commonly encountered anaerobic bacteria grown on brucella base blood agar. We compared the results with those obtained from Robertson's cooked meat medium containing carbohydrates. Some anaerobic isolates produce visible growth on solid medium after 24 hours of incubation and we therefore examined pro-

duction of volatile fatty acid in both solid and liquid media after 24 hours of incubation.

Material and methods

BACTERIA

Previously identified routine clinical isolates of the following species were used: twenty eight *Bacteroides fragilis*; 16 other isolates of the *Bact fragilis* group; six *Clostridium perfringens*, 10 *Clostridium difficile*, 10 other clostridia; seven peptococci, eight peptostreptococci.

MEDIA

Each organism was inoculated on to two blood agar plates (brucella base (Difco): 7% horse blood; vitamin K/haemin 10 mg/l) and into two bottles of cooked meat medium (Robertson's cooked meat; glucose 0.5%; maltose 0.1%; cellobiose 0.1%, Southern Group Laboratories).

CULTURE

The blood plates were incubated at 37°C in an anaerobic chamber (80% nitrogen, 10% hydrogen, 10% carbon dioxide) and the cooked meat medium in a bijou bottle with a screw cap in an incubator at 37°C, without using an anaerobic jar. Extracts of each kind of medium were analysed by gas liquid chromatography after incubation for 24 and 48 hours.

PROCEDURE FOR EXTRACTION

Agar was removed from the well area of plates using

a cork borer 1 cm in diameter. Both media were then acidified using 0.3 ml 50% sulphuric acid. An equal volume of diethyl ether was added to 1.5 ml of cooked meat medium supernatant 1.5 ml agar in a glass tube with a screw top, and or, vortex mixed for 30 seconds and two minutes, respectively. Solvent and aqueous phases were separated by centrifugation for one minute.

CHROMATOGRAPHY

A Perkin-Elmer Sigma 1 chromatograph fitted with a flame ionisation detector and linked to a Perkin-Elmer Sigma 10 computing integrator was used. A 6 ft $\times$ 1/4 inch column packed with 20% neopentyl glycol adipate (Pye-Unicam) on acid washed celite was used. The injection temperature was 160°C and the oven temperature 141°C. The nitrogen carrier gas flow rate was 40 ml/min. The injection volume was 1 μ l.

Results

Extracts prepared from uninoculated media after incubation were tested for volatile fatty acids by gas liquid chromatography. Volatile fatty acids were not detected in any samples of blood agar tested. Small amounts of acetic acid were found in extracts prepared from cooked meat medium.

The concentrations of volatile fatty acids produced by most organisms in either medium after 24 hours of incubation were generally insufficient to be useful in identification. The strains which did produce adequate concentrations of volatile fatty acids

after 24 hours (mostly clostridia) were too few in number to justify reducing the conventional time for incubation.

Although we analysed the volatile fatty acid content of agar taken from the well area of plates, volatile fatty acids can be detected in agar taken from other areas, and the use of pure cultures is therefore imperative.

Figs 1-4 show the production of volatile fatty acids by *Bact fragilis*, *C perfringens*, *C difficile*, and anaerobic Gram positive cocci, respectively. The table shows the production of volatile fatty acids by the other less commonly isolated species.

Discussion

The production of volatile fatty acids by anaerobes as metabolic end products has been exploited for the rapid presumptive diagnosis of anaerobic infections¹ and for the identification of organisms,² as volatile fatty acids are easily detected by gas liquid chromatography. Although this procedure permits presumptive diagnosis of anaerobic infections within 30 minutes of receipt of the specimen, identification of isolates may take several days.

Methods for rapid presumptive identification³ and for rapid definitive identifications of *C difficile*, using gas liquid chromatography, have recently been described. Volatile fatty acids have been detected in extracts prepared from standard³ and modified⁵ selective media and it is suggested that this obviates the need to incubate broth cultures with a subsequent delay in reporting of 24-48 hours.

Fig. 1 Production of volatile fatty acid by *Bacteroides fragilis*

Fig. 2 Production of volatile fatty acid by *Clostridium perfringens*

We found that the same volatile fatty acids are produced in blood agar as in cooked meat medium by *Bact fragilis*, other members of the *Bact fragilis* group, *C difficile*, a range of other clostridia, and anaerobic Gram positive cocci. The concentrations of volatile fatty acids in blood agar were generally less than those in the corresponding cooked meat

Fig. 4 Production of volatile fatty acid by anaerobic gram positive cocci

medium, but this did not create any difficulties in interpreting the profiles produced. Identical volatile fatty acid profiles were not obtained in both media for some strains of species which produce only small amounts ($<1 \mu\text{g/ml}$) of some volatile fatty acids. Minor discrepancies in results such as these do not, however, diminish the usefulness of gas liquid chromatography for identifying such organisms.

We conclude that estimating volatile fatty acids

Fig. 3 Production of volatile fatty acid by *Clostridium difficile*

Table

Organism (n =)	Volatile fatty acids produced in: Cooked meat medium	Blood agar
<i>Bacteroides bivius</i> (1)	A	A
<i>Bacteroides distasonis</i> (5)	A P	A P ib iv
(1)	A P iv	A P ib iv
<i>Bacteroides ovatus</i> (2)	A P	A P iv
(1)	A P iv	A P iv
<i>Bacteroides thetaiotamicron</i> (2)	A P ib iv	A P ib iv
(1)	A p	A P iv
(1)	A p iv	A P ib iv
(1)	A p ib	A P ib iv
<i>Bacteroides vulgatus</i> (1)	A P	A P iv
(1)	A P	A P ib iv
<i>Clostridium cadaverum</i> (1)	A P B	A P B
<i>Clostridium histolyticum</i> (1)	A	A
<i>Clostridium tertium</i> (1)	A B	A B
<i>Clostridium bifermentans</i> (1)	A	A p iv
(1)	A p iv	A p iv ic
<i>Clostridium fallax</i> (1)	A B	A B
<i>Clostridium butyricum</i> (1)	A B	A B
<i>Clostridium sporogenes</i> (1)	A P IB B IV IC	A p ib B IV ic
(1)	A P IB B IV v IC	A p IB b IV ic
(1)	A p IB B IV IC	A p IB b IV ic

Upper case letters: >1 ug/ml; lower case letters: <1 ug/ml.

A: acetic acid; P/p: propionic acid; IB/ib: isobutyric acid; B/b: butyric acid; IV/iv: isovaleric acid; V/v: valeric acid; IC/ic: isocaproic acid.

from a pure culture on solid medium produces results equivalent to the conventional technique and saves both time and media.

References

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