EFFECT OF GLUCOSE CONCENTRATION IN THE GROWTH
MEDIUM UPON NEUTRAL AND ACIDIC FERMENTATION
END-PRODUCTS OF *CLOSTRIDIUM BIFERMENTANS*,
CLOSTRIDIUM SPOROGENES AND
PEPTOSTREPTOCOCCUS ANAEROBIUS

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SUMMARY. *Clostridium bifermentans*, *C. sporogenes* and *Peptostreptococcus anaerobius* were grown in Fastidious Anaerobe Broth with glucose concentrations from 0–1.5% (w/v). Gas chromatographic (GLC) analysis of fermentation end-products revealed that a change in glucose concentration markedly affected fermentation patterns of each microorganism. Increased glucose concentration resulted in stimulation of ethanol production by all strains, and of butanol and butyric acid by *C. sporogenes*. With *C. bifermentans*, there was a parallel increase in ethanol and acetic acid production and decreased formation of propanol and propionic, *iso*-butyric and *iso*-valeric acids. Increased ethanol formation by *P. anaerobius* was accompanied by an increase in acetic acid and a decrease in butan-1-ol production. The greatest incremental change in GLC profiles occurred when glucose concentration was raised from zero to 0.1% (w/v). These data suggest that glucose concentration in a medium for GLC analysis must be rigorously controlled if reproducible results are to be obtained.

INTRODUCTION

Gas chromatographic techniques for assisting classification and identification of microorganisms of clinical significance are now well documented. Such methods are usually based upon analysis of whole cell pyrolysates, specific structural components of cells, or fermentation end-products (Drucker, 1981). Analysis of fermentation end-products of anaerobes has been described in detail by Holdeman, Cato and Moore (1977) and is a routine procedure in many clinical laboratories. The success of fermentation product analysis depends upon the ability of different anaerobes to produce characteristic end-products, in a reproducible manner (Brooks, 1977). Fermentation end-products are formed in primary energy-yielding metabolism and, therefore, ought to be susceptible to modification by catabolite repression of enzyme synthesis, or by modification of enzyme activity (McGilvery, 1979). Fermentation profiles have been shown to be modified by medium composition or incubation time (Mayhew and Gorbach, 1975), by haemin for members of the Bacteroidaceae (Lindner

and Marcelis, 1978), or by fermentable carbohydrate (Lombard, 1979). Glucose is of particular significance in that it may react with other medium constituents; it may also be converted into other metabolic effectors. Saccharolytic organisms preferentially metabolise glucose rather than amino acids, producing straight-chain acids and alcohols. To determine whether glucose concentration in media needs to be rigorously controlled we have examined the effect of different concentrations in Fastidious Anaerobe Broth on the fermentation profiles of three anaerobes.

MATERIALS AND METHODS

Bacteria. *Clostridium bifermentans* strain NCTC506 and *C. sporogenes* strain NCTC532 were obtained from the National Collection of Type Cultures (NCTC), Central PHLS Laboratory, Colindale Avenue, London NW9 5HT. *Peptostreptococcus anaerobius* strain ATCC27337 was obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852, U.S.A. The anaerobes were characterised on the basis of morphology in gram-stained smears, fermentation pattern (Holdeman *et al.*, 1977) after growth in cooked meat medium (Lab-m Ltd, Ford Lane, Salford), and result profile in API 20A (API Ltd, Farnborough, Hants; Hauser and Zabransky, 1976). Additional tests used for *P. anaerobius* were sensitivity to novobiocin (Wren, Eldon and Dakin, 1977) and liquoid (Wideman *et al.*, 1976); API 20A was not used for this organism.

Culture media. The media for fermentation end-product analysis were prepared from Fastidious Anaerobe Broth (FAB; Lab-m). The concentration of glucose in this broth was determined by UV enzymic assay (Boehringer Mannheim, Mannheim, W. Germany). FAB was dispensed in 28-ml glass bottles and supplemented with D-glucose (BDH, Poole, Dorset) to give final concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 and 1.5% (w/v). Bottles were autoclaved at 121°C for 15 min with the caps loose.

Gas chromatography (GLC)

For each strain, triplicate series of 28-ml bottles of FAB were seeded with one drop of culture from cooked-meat medium incubated at 37°C for 48 h. FAB cultures were incubated at 37°C for 72 h, then smears were stained by Gram's method. The cultures were centrifuged and 10-ml of supernatant fluid was acidified with 0.4 ml 50% (w/v) sulphuric acid containing heptanoic acid 1 meq/100 ml as an internal standard. Duplicate ether extractions for GLC analysis were made of all acidified cultures, by mixing 1 ml of culture with 1 ml diethyl ether (Analar; BDH Ltd) after salting-out alcohols with 0.4 g NaCl (Analar).

Ether extracts (2 µl) were injected on to a 1.84 m × 4 mm I.D. glass column of Carbowax 20M TPA 6% on Gas Chrom Q (60–80 mesh) obtained from Phase Separations Ltd, Queensferry, Clwyd. The injections were made with an S8 Autoinjector (Pye Unicam Ltd, Cambridge) and the dual columns were operated in a Pye Unicam 204 gas chromatograph equipped with a Flame Ionisation Detector. Injection temperature was 150°C, detector oven temperature was 250°C; column oven temperature was programmed from 60–160°C at a rate of 8° min, with a final hold of 8 min. Nitrogen flow rate was 40 ml/min; the flow rate for air was 600 ml/min and for hydrogen 40 ml/min. Resultant peaks were integrated using a model 304–50 integrator (Infotronics, Stone, Staffordshire) and the traces recorded on a 10-mV model PM8252 recorder (Phillips, Eindhoven, Netherlands).

For each alcohol and acid of interest, a calibration graph was prepared by extraction and analysis of aqueous solutions of standards by the same methods as the supernatant fluids. Stock solutions of standard alcohols and acids were prepared as described by Holdeman *et al.* (1977) and diluted to give solutions of 0, 0.1, 0.2, 0.5, 1.0, 2.0, 3.5 and 5.0 meq/100 ml for analysis.

RESULTS

The patterns of fermentation end-products of all three strains of bacteria varied

FIG. 1.—Effect of glucose concentration upon (A) volatile acids and (B) alcohols of *C. bifermentans* grown in Fastidious Anaerobe Broth (FAB). ○—○ = acetic acid; △—△ = propionic acid; ●—● = iso-butyric acid; ■—■ = iso-caproic acid; ○—○ = ethanol; △—△ = propanol.

directly with glucose concentration in FAB; the maximum effect occurred between 0.1–0.4% (w/v). *C. bifermentans* produced more acetic acid relative to other acids (fig. 1A) and the ratio of ethanol to propanol was markedly increased when the glucose concentration was raised to 0.1% (w/v) (fig. 1B). These modified profiles were very evident when the gas chromatograms were inspected. With *C. sporogenes*, the alterations in amounts of acetic and butyric acids were particularly obvious (fig. 2A). Such changes were mirrored by alterations in proportions of the corresponding straight chain alcohols, ethanol and butan-1-ol (fig. 2B). *P. anaerobius* also produced

FIG. 2.—Effect of glucose concentration upon (A) volatile acids and (B) alcohols of *C. sporogenes* grown in FAB. ○—○ = acetic acid; □—□ = butyric acid; ●—● = iso-butyric acid; ○---○ = ethanol; Δ---Δ = propanol; ●---● = iso-butanol; □---□ = butan-1-ol.

more acetic acid relative to other acids as glucose concentration was increased (fig. 3A). There was an associated increase of the corresponding alcohol, ethanol (fig. 3B) with a fall in the proportion of butan-1-ol to a very low level in FAB that contained as little as 0.1% glucose (w/v). Coefficients of variation for each peak analysed indicated that experimental variation was very low; values ranged from 1–6% acetic acid produced by *C. bifermentans* in the various glucose concentrations.

DISCUSSION

The effects observed with *C. bifermentans* indicate the considerable influence of

FIG. 3.—Effect of glucose concentration upon (A) volatile acids and (B) alcohols of *P. anaerobius* grown in FAB. ○—○ = acetic acid; ●—● = *iso*-butyric acid; ■—■ = *iso*-caproic acid; ○---○ = ethanol; □---□ = butan-1-ol.

glucose concentration on end products. With raised glucose, both acetic acid and ethanol concentrations rise. The latter compounds are produced as alternative 2-carbon fermentation products. If the cell's ratio of $\text{NAD}^+ : \text{NADH}$ is decreased, it may be restored by reduction of acetyl-CoA to ethanol; otherwise formation of acetic acid will permit synthesis of ATP (Doelle, 1969). Production of branched chain acids and alcohols arises primarily from reductive deamination of *iso*-leucine, leucine and valine (Elsden and Hilton, 1978). Deamination may be inhibited by glucose, being replaced by decarboxylation following a fall in pH.

Similar biochemical arguments may be applied to the data obtained with *C.*

sporogenes. With this organism, increase in glucose concentration stimulates an increase in the proportions of acetic and butyric acids and of ethanol and butan-1-ol. Acetic and butyric acids can both arise from catabolism of histidine or cysteine (Loesche and Gibbons, 1968), threonine (Elsden and Hilton, 1978), aspartate or lysine (Stadtman, 1963) or glutamate (Buckel and Barker, 1974). However, acetic acid can also arise from glucose *via* pyruvate. Similarly, butyric acid can readily be formed from acetyl-CoA derived from pyruvate. Two acetyl residues are condensed to yield acetoacetyl-CoA which undergoes β -reduction to butyric acid and ultimately to butan-1-ol.

The interpretation of data for *P. anaerobius* is slightly complicated by the many different effects of glucose upon microorganisms, e.g., inhibition of growth (Shanson, 1978), reduction in viability (Ganguli, Turton and Tillotson, 1982), and modification of biomass and enzyme production (Greenman, Holland and Cunliffe, 1981). Acetic acid and ethanol are proportionately increased by glucose but butan-1-ol formation is suppressed. Even in the absence of glucose, little butyric acid was found. The source of butan-1-ol is not clear. The production of *iso*-caproic acid in all concentrations of glucose possibly indicates constitutive enzymes for catabolism of leucine or *iso*-leucine (Elsden and Hilton, 1978).

The relevance of the above findings for the diagnostic laboratory is that glucose concentrations in commercial media range from as little as 0.01 g/L in FAB to 10 g/L in Peptone Yeast-Extract Glucose Broth. When cooked meat media of four different manufacturers were recently analysed, the glucose concentrations ranged from 0.02–0.58 g/L (unpublished data). Such variations would result in major quantitative differences in fermentation profiles with any of the anaerobes examined in this study. One solution would be to use a medium with a very low glucose content, e.g., FAB. Another solution would be to follow the practice of the VPI anaerobe laboratory and use media containing 1% (w/v) glucose (Holdeman *et al.*, 1977); at this level trace amounts in medium constituents are unlikely to have a significant effect upon the total concentration of glucose available. The two disadvantages of the latter approach are the lethal effects of excess glucose for some anaerobes and a possible inhibition of catabolism of amino acids to yield branched chain alcohols and acids.

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