SHORT COMMUNICATIONS

Cytochrome Synthesis by *Bifidobacterium* during Growth in Media Supplemented with Blood

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INTRODUCTION

Van der Wiel-Korstanje & Winkler (1970) reported that bifidobacteria form dark brown colonies on reinforced clostridial agar (RCA, Oxoid) supplemented with glucose, horse blood and iron salts (China blue). Subsequent work showed that iron salts in the growth medium were not essential for the formation of the brown pigment. However, omission of glucose or blood (or lysed red cells) from the growth medium resulted in the formation of unpigmented colonies. In addition, several *Bifidobacterium* strains (25% of our isolates) reduced nitrate after growth in the presence of lysed red cells, glucose and nitrate. The present paper deals with the formation of cytochromes by bifidobacteria growing on blood-containing media, and the involvement of cytochromes in nitrate reduction.

METHODS

Strains of *Bifidobacterium*, isolated from normal adult faeces as described previously (van der Wiel-Korstanje & Winkler, 1970), were identified morphologically and biochemically as described by Reuter (1963) and Mitsuoka (1969), and shown to contain fructose-6-phosphate phosphoketolase (Scardovi & Trovatelli, 1965; de Vries, Gerbrandy & Stout-hamer, 1967). A nitrate-reducing strain, identified as *Bifidobacterium longum*, was selected for further investigations.

Bifidobacterium longum was cultured in 100 ml bottles completely filled with basal medium which contained (g/l distilled water): proteose peptone (Difco), 15; yeast extract (Difco), 5; meat extract (Oxoid), 3; NaCl, 2.5; Na₂HPO₄ · 2H₂O, 2.5; cysteine, 0.5; final pH 7.1. To this was added one of the following supplements: (i) glucose (20 g/l) and lysed sheep red cells (30 g/l); (ii) glucose, lysed red cells and KNO₃ (1 g/l); (iii) glucose and KNO₃; (iv) lysed red cells and KNO₃.

After 48 h at 37 °C bacteria were centrifuged, washed twice with sodium-potassium phosphate buffer (pH 6.8) and resuspended in this buffer to a suitable extinction. Dry weights of the bacteria were determined after lyophilization. Dithionite-reduced versus oxygen-oxidized difference spectra were recorded between 400 and 650 nm using an Aminco Chance recording spectrophotometer (American Instruments Co. Inc., Washington, U.S.A.).

The amount of cytochrome *b* was calculated from $\epsilon = 17500$ (Deeb & Hager, 1964).

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Fig. 1. Cytochrome spectra of *Bifidobacterium longum* grown with and without lysed red cells and glucose. The numbers I, II, III and IV refer to the media mentioned under Methods. Suspensions I and II contained about 0.6 mg bacterial dry wt/ml. Suspensions III and IV contained about 10 mg bacterial dry wt/ml.

RESULTS AND DISCUSSION

Suspensions of *Bifidobacterium longum* grown with lysed red cells and glucose were brown and contained appreciable quantities of cytochrome *b* (peaks at 529 and 560 nm) and cytochrome *d* (peak at 622 nm) (Fig. 1, spectrum I). After growth with lysed red cells, glucose and nitrate large amounts of cytochrome *a* (peak at 586 nm) and cytochrome *b* were present (Fig. 1, spectrum II). Such bacteria showed active nitrate reduction. When the red cells or glucose had been omitted from the growth medium, bacterial suspensions were white, cytochromes were absent (Fig. 1, spectra III and IV) and the bacteria did not reduce nitrate.

Bifidobacterium longum can therefore synthesize cytochromes provided that glucose and blood factors are present in the growth medium. The cytochrome content of the bacteria (up to 6 μmol cytochrome *b*/g bacterial dry wt) was much higher than the values found for facultative and aerobic micro-organisms. For instance, Kröger & Dadak (1969) found 0.14 μmol cytochrome *b*/g protein in *Bacillus megaterium*. The high cytochrome content might explain the brown colour of suspensions and colonies grown with blood.

Formation of cytochromes in haem-containing media has been reported for other lactic acid bacteria. Bryan-Jones & Whittenbury (1969) presented evidence for a cytochrome-containing electron transport system in haematin-grown *Streptococcus faecalis*. Kaars Sijpesteijn (1970) demonstrated cytochromes in *S. lactis* and *Leuconostoc mesenteroides* after growth with haemin.

The function of cytochromes in the metabolism of bifidobacteria seems to be associated with nitrate reduction. Nitrate-reducing strains only reduced nitrate after growth in the presence of lysed red cells and glucose. The ability to reduce nitrate disappeared after a rapid series of subcultures in haem-free media. Nitrate reduction and cytochrome synthesis reappeared immediately after inoculation into a medium with lysed red cells and glucose. Cytochrome *a* seems to be closely linked to nitrate reduction because this cytochrome is formed only in the presence of nitrate. The two other cytochromes may have another function because they are also present in strains not reducing nitrate.

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