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Anaerobiospirillum species isolated from humans with diarrhoea

H MALNICK, MEM THOMAS,* H LOTAY,* M ROBBINS

*From the National Collection of Type Cultures and Epidemiological Research Laboratory, Central Public Health Laboratory, London NW9 5HT, and the *Department of Clinical Microbiology, University College Hospital, London WC1E 6AU*

SUMMARY Flagellated anaerobic motile spiral bacteria were isolated from the faeces of two patients with diarrhoea. They were recovered by the microaerophilic culture method used to detect campylobacters but demanded anaerobic conditions for subculture. Electron microscopy and other investigations showed them to be closely related to *Anaerobiospirillum succiniproducens* first described in beagle dogs and subsequently in three humans with bacteraemia.

Anaerobic spiral bacteria in the human bowel have not often been reported. When found, such organisms have been of two types, spirochaetes with axial fibrils[1–7] and non-spirochaetes without visible axial fibrils.[3,8] We here report the isolation of relatively anaerobic flagellated spiral bacteria from the faeces of two patients with diarrhoea.

Material and methods

The organisms were isolated during the routine diagnostic examination of faecal specimens. The first patient (A30) was a 41-year-old man who had recurrent pain and loose stools, the second (A142) was a child who attended a local Day Nursery. Rotavirus, *Shigella, Salmonella* and *Campylobacter* species were not found in either patient. Faeces from 34 child contacts of A142 were examined, but the spiral organism was found in this one case only. Six other children were excreting *Shigella sonnei.*

The two spiral organisms were designated A30 and A142 respectively.

CULTURE METHODS

The methods in routine use for the recognition of intestinal pathogens included a selective medium for campylobacters containing vancomycin, polymyxin and trimethoprim[9] which was incubated for 48 h at 43°C employing a BBL Campylobacter ("Campypak") sachet in a jar to secure a microaerophilic atmosphere.

Both strains of spiral bacterium were initially isolated by this method, but anaerobic incubation was required for regular successful subculture. Oxygen and temperature tolerance were investigated in parallel on 5% horse blood agar, chocolate agar, lysed blood agar and buffered charcoal yeast extract agar (BCY)[10] using an evacuation system in an anaerobic jar, incubated at a range of temperatures between 30°C and 45°C. Ninety per cent hydrogen and 10% carbon dioxide gas were utilised as a basic atmosphere to obtain a comparison between oxygen concentrations of 5%, 4% and 3·5% and strict anaerobiosis.

FERMENTATION AND BIOCHEMICAL TESTS

Cell suspensions in distilled water were made from 24-hour anaerobic cultures on blood agar and used for inoculation. The methods of Cowan and Steel[11] were used with the following modifications; skimmed milk powder was used for testing casein hydrolysis; sodium nitrate for nitrate reduction and 0·4% starch for starch hydrolysis. The blue catalase test,[12] which traps bubbles, was compared with the standard method. The basal medium for testing acid production from carbohydrates and glucose fermentation products was peptone yeast broth.[13] The end products of glucose fermentation were analysed by gas liquid chromatography on a Pye Unicam Series 204 Chromatograph.

ANTIBIOTIC SENSITIVITY

The disc method was used on blood agar and BCY incubated anaerobically at 37°C and 43°C. A strain of *Campylobacter jejuni* freshly isolated from a blood culture was tested in parallel on media incubated microaerophilically.

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CELL MORPHOLOGY

Gram-stained impression preparations were examined after 24 and 48 h culture on 5% horse blood agar and BCY in microaerophilic and anaerobic atmospheres at 37°C and at 43°C. For electron microscopy (EM), cultures on BCY medium incubated anaerobically at 37°C for 24 and 48 h were scraped and suspended in 2% formaldehyde solution. The bacterial cells were stained with 1% phosphotungstic acid and then examined under an AEI EM6B electron microscope. The wavelength and amplitude of the spirals seen were measured on EM photographs and compared with those recorded for typical *Anaerobiospirillum succiniproducens*[14] and *Campylobacter jejuni*[15] strains (Table 1).

Results

MORPHOLOGY

The initial cultures A30 and A142 were obtained on campylobacter selective medium incubated microaerophilically at 43°C for 48 h. They resembled *Campylobacter jejuni* in colonial and microscopic appearance but were oxidase-negative and were difficult and often impossible to subculture microaerophilically. However they grew well after 24 h in an anaerobic atmosphere on 5% horse blood agar, chocolate agar, lysed blood agar and BCY medium, but not on nutrient agar. The temperature tolerance extended from 32°C to 43°C. Colonies on blood agar after 24 hours at 37°C were 0·5 to 1 mm in diameter, translucent and non-haemolytic, with some delicate spreading or swarming peripheral growth which was more marked at 43°C than at 37°C (Fig. 1). Impression films showed Gram-negative spiral organisms 3–5 μm long and 0·5 μm wide (Fig. 2), after 24 hours incubation but at 48 hours the cells had elongated up to 34 μm with as many as 20 regularly spaced spirals (Fig. 3). Older cultures became progressively more fragmented and coccoid. Dark field microscopy revealed spiral motile cells which revolved around their long axes. Electron microscopy showed these cells to have bipolar tufts of multiple flagellae (Figs. 4 and 5). Organism A30 had a spiral wavelength of 1·4–2 μm with an amplitude of 0·23–0·35 μm, while A142 had a wavelength of 1·7–2·2 μm and an amplitude of 0·2–0·65 μm (Table 1).

Table 1 *Characteristics of spiral organisms A30 and A142 compared with those of Anaerobiospirillum succiniproducens*[14] *and Campylobacter jejuni*[15]

	A30	*A142*	*A succiniproducens*	*C jejuni*
Acid from:				
Fructose	+	–	+	–
Glucose	+	+	+	–
Inositol	–	–	–	–
Lactose	–	–	+	–
Maltose	–	–	v	–
Mannitol	–	–	–	–
Raffinose	–	–	+	–
Salicin	–	–	–	–
Sorbitol	–	–	–	–
Sucrose	–	–	+	–
Trehalose	+	–	v	–
Casein	–	–	NT	–
Oxidase	–	–	–	+
Catalase	–	–	–	+
Blue catalase test	trace	trace	trace	+
Glucose fermentation: acetic & succinic acid as major end products	+	+	+	–
Subculture growth:				
anaerobic	+	+	+	–
aerobic	–	–	–	–
3·5–5% O_2	–	–	–	+
Lecithinase	–	–	v	–
Methyl red	–	–	NT	–
Nitrate reduction	–	–	–	+
ONPG	–	–	NT	–
Phosphatase	–	–	NT	NT
Starch hydrolysis	–	–	v	–
Temperature:				
Maximum	43°C	43°C	40°C approx	43°C
Minimum	32°C	32°C	25°C approx	37°C approx
Urea hydrolysis	–	–	–	–
Spiral wavelength (μm)	1·4–2	1·7–2·2	1·3–1·7	0·9–1·3
Amplitude (μm)	0·23–0·35	0·2–0·65	0·9–1·1	0·35–0·6

v = variable, NT = not tested.

Fig. 1 *Feathery swarming growth of Anaerobiospirillum on surface of blood agar after 48 hours anaerobic incubation ×2.*

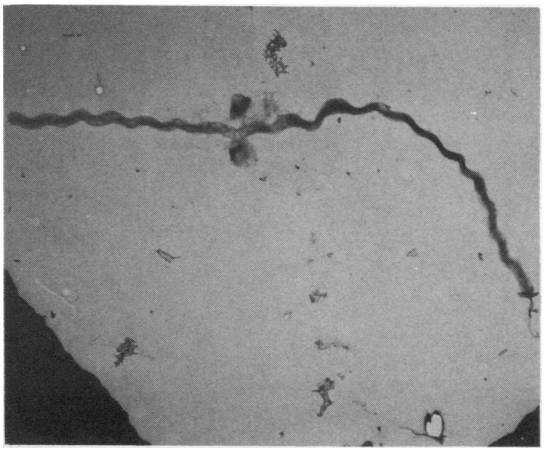

Fig. 3 *Electron micrograph of Anaerobiospirillum cultured on BCY for 48 hours, showing many spirals ×2250.*

BIOCHEMICAL AND FERMENTATION TESTS

The results are summarised in Table 1 which includes a comparison with some reactions characteristic of type strains of *Anaerobiospirillum succiniproducens* and of *Campylobacter jejuni*. Oxidase tests were negative. Conventional catalase tests were also negative, but a trace reaction was obtained consistently with the more sensitive blue peroxide catalase test[12] with which the type strain of *A succiniproducens* also gave a trace reaction. Gas liquid chromatography detected acetic and succinic acids as major end products of glucose fermentation by both A30 and A142, as by the type species *A succiniproducens;* but not by *C jejuni.*

The two spiral organisms A30 and A142 resembled each other and *A succiniproducens* in cultural requirements and morphology but differed slightly in biochemical tests. They differed culturally, morphologically and biochemically from campylobacters.

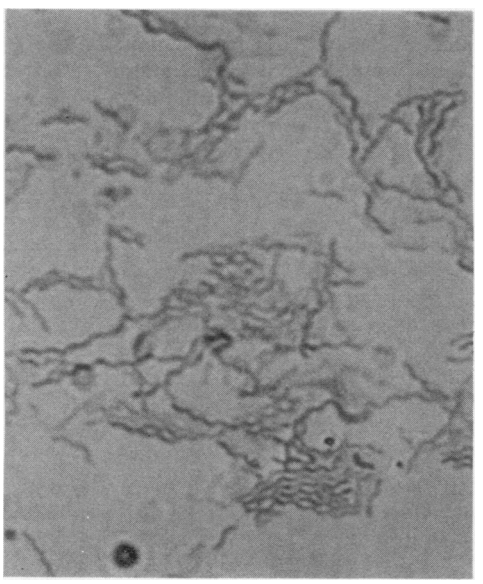

Fig. 2 *Impression film of Anaerobiospirillum cultured anaerobically on blood agar for 48 hours, stained Gram ×1600.*

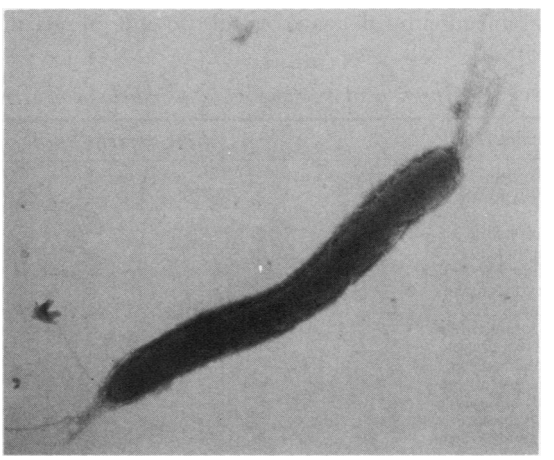

Fig. 4 *Electron micrograph of Anaerobiospirillum cultured on BCY for 24 hours, showing bipolar tufts of flagella ×13 500.*

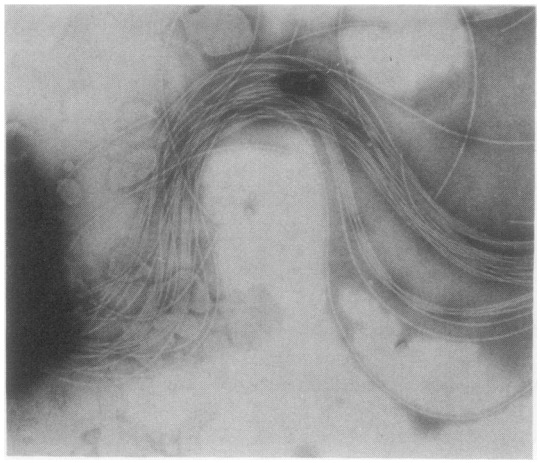

Fig. 5 *Electron micrograph of Anaerobiospirillum cultured on BCY, showing flagella tuft at higher magnification ×29 250.*

ANTIMICROBIAL SENSITIVITY

The results of disc sensitivity tests are shown in Table 2. They were the same on BCY and blood agar plates at 37°C and 43°C. Apart from a difference in the degree of resistance to gentamicin, A30 and A142 were alike. They were, unlike *C jejuni,* more sensitive to penicillin than to erythromycin. Although sensitive to the standard 300 unit polymyxin disc they grew on a campylobacter selective medium[9] which contained polymyxin 2·5 units per ml.

Discussion

There have not been many reports of human intestinal infection by elongated spiral bacteria. In recent descriptions the diagnosis has been achieved by histological examination of biopsy material and by electron microscopy.[1-8 16] Most of these reports describe bacteria with axial filaments, confirming that they belong to the Family *Spirochaetaceae.* Takeuchi *et al*[3 17] however, observed an organism with single bipolar flagella in colonic epithelium from humans and from rhesus monkeys, but did not indicate whether it had been cultured. Two reports concern long spiral bacteria which were successfully cultured; one of these showed neither axial fibrils nor flagella[8] and the other was not examined by electron microscopy.[18] In 1976 Davis *et al*[14] reported the isolation of an *Anaerobiospirillum* species from the mouth and faeces of beagle dogs and proposed the type species *A succiniproducens.* This organism has since been recovered from blood cultures in three febrile adult human patients.[19 20] Like *A succiniproducens,* the two anaerobic spiral organisms we describe have bipolar tufts of flagella, but they differ slightly from the type strain and from each other in their pattern of carbohydrate fermentation. On the basis of tests carried out so far we conclude that A30 and A142 are *Anaerobiospirillum* species, but not necessarily *A succiniproducens.* Although they were isolated from the faeces of patients suffering from diarrhoea there is insufficient evidence on which to ascribe pathogenicity. It may however prove worthwhile to seek such bacteria in future. Although essentially anaerobic and intolerant of oxygen on subculture, both A30 and A142 were initially isolated microaerophilically on a campylobacter selective medium incubated at 43°C for 48 h and the strains were moderately sensitive to metronidazole. At that stage the colonial form and the appearance of Gram-stained bacteria closely resembled those of a campylobacter although some

Table 2 *Sensitivity of A30 and A142 to antimicrobial agents*

Antimicrobial	*Disc content (IU/μg)*	*A30*	*A142*	*Campylobacter jejuni*†
Penicillin	2 IU	S	S	R
*Polymyxin	300 IU	S	S	R
Ampicillin	10	S	S	S
Cephalexin	30	S	S	R
Chloramphenicol	10	S	S	S
Erythromycin	10	M	M	S
Fusidic acid	10	R	R	R
Gentamicin	10	S	M	S
Metronidazole	5	M	M	R
Nalidixic acid	30	M	M	S
Nitrofurantoin	50	S	S	S
Sulphafurazole	100	S	S	S
Tetracycline	10	S	S	S
*Trimethoprim	1·25	R	R	R
*Vancomycin	35	R	R	R

S = sensitive; M = moderately sensitive; R = resistant.
*Component of the campylobacter selective medium used for isolation.
†Strain from a blood culture.

Table 3 *Typical characteristics of non-aerobic spiral bacteria*[21 22]

	Flagella	*Growth at* 25°C	35°C	40°C	*Oxidase*	*Catalase (conventional)*
Microaerophils						
Campylobacter spp	1 polar	v	+	v	+	+
Spirillum volutans	bipolar tuft	+	+	−	+	+
Anaerobes						
Anaerobiospirillum spp	bipolar tuft	v	+	+	−	−
Desulfovibrio spp	polar tuft	+	+	+	−	−
Wolinella spp	polar tuft	−	+	−	−	−
Selenomonas spp	lateral	v	+	v	−	−
Butyrivibrio spp	1 polar	−	+	+	−	−
Succinivibrio spp	1 polar	−	+	−	−	−
Anaerobic spirochaetes	axial only	v	+	v	−	−

v = variable.

spiral cells were very long.

Campylobacter jejuni isolated from faeces at 43°C are characteristically oxidase and catalase positive by conventional methods and most strains are fully sensitive to erythromycin, so the negative results we obtained with these three tests indicated the need for further study. Biochemical tests were not found very helpful until an identification to Family level was achieved. At present, as is seen in Table 3, electron microscopy is the best way to discriminate between the known groups of spiral bacteria.

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Requests

Mr H Malnick, National Collection of Type Cultures, Central Public Health Laboratory, Colindale, London NW9 5HT would be grateful to receive strains or information about similar spiral organisms isolated elsewhere

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Requests for reprints to: Dr Mair EM Thomas, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, England.