Bacteroides ureolyticus (NTU) medium for the selective recovery of *Bacteroides gracilis*

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Summary. *Bacteroides gracilis* is a gram-negative anaerobic bacillus which requires formate and fumarate for growth; it has been implicated in periodontal disease and serious infections of the head and neck. In this study, *Bacteroides ureolyticus* (NTU) medium was tested for its ability to allow the growth of *B. gracilis* and other formate-fumarate requiring gram-negative anaerobes and to enable the recovery of these organisms from clinical specimens. All reference strains grew on NTU medium with the exception of *Wolinella recta* and formate-fumarate requiring organisms were isolated from 18 of 20 samples of subgingival dental plaque from patients with chronic periodontitis. *B. gracilis* was the commonest species isolated (14 of the 29 isolates); *B. ureolyticus* was not found.

Introduction

Bacteroides gracilis requires formate and fumarate for growth and since the restriction of the genus *Bacteroides* to the fragilis group¹ its move to a new genus is pending. *B. gracilis* has been implicated in serious infections of the head and neck² and is frequently isolated from subgingival plaque from patients with chronic periodontitis.³ A selective and differential medium (NTU; Lab M, Bury) is available for *B. ureolyticus*,⁴ another formate-fumarate requiring species; it contains nalidixic acid 10 mg/L and teicoplanin 20 mg/L as selective agents and an indicator system for urease activity. A similar medium containing nalidixic acid 8 mg/L and teicoplanin 32 mg/L has been shown to be useful for the selective isolation of *B. gracilis*.⁵ Therefore, NTU medium was tested for its ability to support the growth of *B. gracilis* and other formate-fumarate dependent bacteria and to enable their isolation from subgingival dental plaque.

Materials and methods

Reference strains of formate-fumarate requiring bacteria

B. gracilis ATCC 33236, *Wolinella curva* ATCC 35224 and *W. recta* ATCC 33238 were obtained from the American Type Culture Collection (Rockville, MD) and *B. ureolyticus* NCTC 10941 and *Campylobacter concisus* NCTC 11485 from the National Collection of Type Cultures (Colindale Avenue, London).

Suspensions of the bacteria were prepared in

reduced transport medium⁶ and inoculated on to NTU medium and Fastidious Anaerobe Agar (FAA; Lab M) by means of a spiral plater (Don Whitley Scientific, Shipley). The plates were incubated for 96 h in an anaerobic cabinet (Don Whitley Mk III) in an atmosphere of N₂ 80%, H₂ 10%, CO₂ 10% at 37°C. After incubation the viable counts (number of cfus) on the two media were compared.

Plaque samples

Subgingival plaque was collected by means of a small spoon excavator from periodontal pockets > 6 mm deep from 20 patients with chronic periodontitis. Samples were placed in 2 ml of reduced transport medium and taken to the laboratory where they were dispersed by vortex mixing with 200 µl of glass beads 150 µm in diameter. Ten-fold dilutions were prepared in reduced transport medium, spiral plated on FAA and NTU, and incubated in the anaerobic cabinet for 96 h. After incubation, the number of cfus were counted. In addition, the NTU plates were examined and the morphology and numbers of the different colony types were recorded. Two colonies of each type were sub-cultured on to FAA and incubated for 48 h. Gram-stained films were made from all isolates and they were investigated for atmospheric requirements. Any small gram-negative anaerobic bacilli were tested for the ability of formate-fumarate to stimulate their growth in broth culture.⁷ Isolates other than formate-fumarate requiring organisms were identified to genus levels by standard methods.⁸

Formate-fumarate requiring organisms were tested for production of oxidase and urease, and for motility as described previously.⁹ Definitive identification was achieved with SDS-PAGE protein profiles.⁹ Briefly, protein profiles were obtained on gradient SDS 10–

15% gels run on the PhastSystem (Pharmacia, Milton Keynes). These were scanned with a densitometer (Chromoscan 3; Joyce Loebel, Gateshead) and the data were analysed by means of the PAGETAX software, developed from that described by Jackman,¹⁰ run on a microcomputer (Apricot Xen-i 386/30, Birmingham).

Results

The results obtained with the reference strains on NTU medium, expressed as a percentage of FAA counts, were (mean of three replicates): *B. gracilis* ATCC 33236, 94.4; *B. ureolyticus* NCTC 10941, 98.9; *C. concisus* NCTC 11485, 97.6; *W. curva* ATCC 35224, 94.6. *W. recta* ATCC 33238 did not grow on NTU medium.

Eighteen of the 20 samples of subgingival plaque yielded formate-fumarate requiring organisms (table). *B. gracilis* was detected in 11 samples and constituted 6.2% of the flora when present. *W. recta*, *W. curva* and *C. concisus* were also found but *B. ureolyticus* was not isolated. Six strains were assigned to three protein profile clusters distinct from clusters that included a type strain of a named species. These strains were non-motile and oxidase- and urease-negative.

NTU medium enabled the isolation of formate-

fumarate requiring organisms in pure culture from nine of the subgingival plaque specimens. The "contaminants" found on the other plates were gram-negative anaerobes belonging to the genera *Bacteroides*, *Fusobacterium* and *Veillonella* and capnophilic *Capnocytophaga* species. Contaminating organisms were easily distinguished by colony morphology alone.

Discussion

Bacteroides ureolyticus (NTU) medium has been shown to be an effective selective medium for *B. gracilis*, as might have been predicted from its similarity to a medium described for the selective recovery of *B. gracilis*.⁵ Contaminating organisms were few, and easily distinguished from the target species. Furthermore, with samples of subgingival plaque, it proved valuable for the selective isolation also of *C. concisus* and *W. curva*. The type strain of *W. recta* failed to grow on NTU medium but some fresh isolates of this species were obtained from cultures of subgingival plaque. However, previous studies have shown that *W. recta* is the most common member of the formate-fumarate group encountered in subgingival plaque.^{11, 12} It seems likely, therefore, that one of the selective agents incorporated into the medium is present at around the MIC for this species,

Table. Formate-fumarate requiring and contaminating organisms isolated on NTU medium from subgingival plaque samples

Sample no.	Species isolated	Percentage of total count on FAA	Contaminating organisms
1	UFF1	1.84	...
	UFF2	0.74	
	UFF3	0.18	
2	<i>B. gracilis</i>	27.38	<i>Fusobacterium</i> sp.
	<i>C. concisus</i>	2.59	
3	<i>B. gracilis</i>	6.15	<i>Bacteroides</i> sp.
4	—	—	<i>Fusobacterium</i> sp.
5	<i>C. concisus</i>	0.87	<i>Fusobacterium</i> sp.
6	<i>B. gracilis</i>	9.22	<i>Capnocytophaga</i> sp.
7	<i>B. gracilis</i>	2.50	...
8	<i>B. gracilis</i>	0.87	...
9	<i>W. recta</i>	0.45	<i>Veillonella</i> sp.
10	<i>B. gracilis</i>	4.41	<i>Fusobacterium</i> sp.
11	<i>W. recta</i>	7.21	...
	UFF2	7.21	
12	<i>B. gracilis</i>	12.06	...
13	<i>B. gracilis</i>	2.65	...
14	UFF3	0.74	...
15	<i>B. gracilis</i>	0.59	<i>Fusobacterium</i> sp.
16	UFF3	21.43	...
17	—	—	<i>Fusobacterium</i> sp.
18	<i>W. recta</i>	0.58	<i>Capnocytophaga</i> , <i>Fusobacterium</i> , <i>Veillonella</i> spp.
	<i>C. concisus</i>	0.20	
	<i>B. gracilis</i>	0.25	
19	<i>W. curva</i>	2.42	...
	<i>W. recta</i>	1.21	
20	<i>B. gracilis</i>	2.00	<i>Capnocytophaga</i> , <i>Veillonella</i> spp.

UFF, unidentified formate-fumarate requiring organisms.

..., formate-fumarate requiring species isolated in pure culture.

allowing the growth of only a subset of organisms. It might prove possible to modify the medium to enable full and selective isolation of *W. recta*. In common with previous studies,^{9,13} a small but significant number of isolates did not correspond to established species. Further taxonomic studies are required to investigate their relationship with established taxa.

NTU medium, in its present formulation, allows the easy detection of *B. ureolyticus* (pink colonies). A further test for motility allows the differentiation of *B. gracilis* (non-motile) from the motile *Wolinella* and *Campylobacter* spp. The use of this medium should greatly facilitate further studies of the role of these organisms in disease at oral and non-oral sites.

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