Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis of Cell-surface Proteins as an Aid to the Identification of the *Bacteroides fragilis* Group

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The outer-membrane complexes from 40 strains of *Bacteroides*, representing eight of the species included in the *Bacteroides fragilis* group, were released by EDTA treatment. The component polypeptides were examined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis on slab gels. Within a species (biotype) the patterns produced indicated marked similarities in the structures of the surface proteins among the strains examined. The patterns produced by strains belonging to different species, however, showed fewer similarities. An unknown organism could therefore be identified to species level using this technique and a few selected biochemical tests.

INTRODUCTION

Bacteroides fragilis, *B. vulgatus*, *B. distasonis*, *B. thetaiotaomicron*, *B. eggerthii*, *B. ovatus*, *B. uniformis* and *B. splanchnicus* are species included in the *B. fragilis* group. They are characterized as being obligately anaerobic, non-pigmented, bile-tolerant, glucose-fermenting, Gram-negative rod-shaped bacteria. These organisms are frequently isolated from clinical specimens, predominantly from wounds, intra-abdominal infections, the blood and perirectal or pelvic sites (Finegold, 1977; Willis, 1977; Wren *et al.*, 1977). *Bacteroides fragilis*, a minor component of the faecal flora, is the member of the group most commonly isolated from infections, whereas *B. vulgatus*, *B. thetaiotaomicron* and *B. uniformis* are predominantly faecal isolates.

After an organism has been identified as a member of the family Bacteroidaceae by culture and microscopic examination, and is distinguished from the fusobacteria by gas-liquid chromatography of the volatile fatty acid metabolic products, at least eight, sometimes variable, biochemical tests have to be performed for identification to species level (Holdeman *et al.*, 1977).

Recently, polyacrylamide gel electrophoresis (PAGE) of cellular proteins has been employed in attempts to sub-classify the *Bacteroides* genus. Strom *et al.* (1976) used discontinuous gradient PAGE and examined proteins solubilized by sonication of whole cells of several species of Bacteroidaceae. Swindlehurst *et al.* (1977) used the SDS (sodium dodecyl sulphate)–PAGE procedure to examine members of the species *B. melaninogenicus*; these workers used hot SDS extracts of whole cells. It was possible to identify members of the Bacteroidaceae to species, subspecies and strain levels.

The present study evaluates the use of SDS–PAGE in examining the cell-surface proteins released from the bacteria by controlled treatment with EDTA at 45 °C followed by mild ultrasonic disruption. When this is used in conjunction with a few selected biochemical tests it becomes a relatively rapid, simple aid to identifying the species within the *B. fragilis* group.

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Table 1. *Strains of Bacteroides examined by SDS-PAGE and results of selected biochemical tests*

Species	Strain no.	Source	Indole pro- duction	Fermentation of:			
				Rha	Tre	ManOH	Suc
<i>B. fragilis</i>	NCTC 9344	NCTC	—	—	—	—	NT
	NCTC 8560	NCTC	—	—	—	—	NT
	NCTC 10581	NCTC	—	—	—	—	NT
	NCTC 10584	NCTC	—	—	—	—	NT
	GNAB 4	Wound swab RIE	—	—	—	—	NT
	GNAB 8	Wound swab RIE	—	—	—	—	NT
	GNAB 49	Skin RIE	—	—	—	—	NT
	GNAB 101	Wound swab WGHE	—	—	—	—	NT
	GNAB 102	Wound swab WGHE	—	—	—	—	NT
	GNAB 104	Wound swab WGHE	—	—	—	—	NT
	GNAB 105	Wound swab WGHE	—	—	—	—	NT
<i>B. vulgatus</i>	NCTC 10583	NCTC	—	+	—	—	NT
	GNAB 25	Faeces	—	+	—	—	NT
	GNAB 30	Faeces	—	+	—	—	NT
	GNAB 31	Faeces	—	+	—	—	NT
	GNAB 34	Faeces	—	+	—	—	NT
	GNAB 35	Faeces	—	+	—	—	NT
	GNAB 37	Faeces	—	+	—	—	NT
	GNAB 38	Faeces	—	+	—	—	NT
	GNAB 44	Faeces	—	+	—	—	NT
	GNAB 107	Wound swab WGHE	—	+	—	—	NT
	GNAB 120	Wound swab WGHE	—	+	—	—	NT
<i>B. distasonis</i>	ATCC 8503	ATCC	—	+	+	—	NT
	GNAB 22	Faeces	—	+	+	—	NT
	GNAB 27	Faeces	—	+	+	—	NT
	GNAB 39	Faeces	—	+	+	—	NT
<i>B. thetaiota- omicron</i>	NCTC 10582	NCTC	+	+	+	—	NT
	GNAB 1	Wound swab RIE	+	+	+	—	NT
	GNAB 2	Wound swab RIE	+	+	+	—	NT
	GNAB 7	Wound swab RIE	+	+	+	—	NT
<i>B. eggerthii</i>	NCTC 11155	NCTC	+	+	—	—	—
	GNAB 21	Faeces	+	+	—	—	—
	GNAB 24	Faeces	+	+	—	—	—
	GNAB 41	Faeces	+	+	—	—	—
<i>B. uniformis</i>	ATCC 8492	ATCC	+	+	—	—	+
	VPI 11227	VPI	+	—	+	—	+
	GNAB 18	Wound swab RIE	+	+	—	—	+
	VPI 11368	VPI	+	+	—	—	+
<i>B. ovatus</i>	ATCC 8483	ATCC	—	+	+	+	+
<i>B. splanchnicus</i>	NCTC 10825	NCTC	+	—	—	—	NT

+, Positive; —, negative; NT, not tested.

Non-standard abbreviations: VPI, Virginia Polytechnic Institute, Blacksburg, Virginia, U.S.A.; RIE, Royal Infirmary, Edinburgh; WGHE, Western General Hospital, Edinburgh; GNAB, Lab. strain no.

Rha, Rhamnose; Tre, trehalose; ManOH, mannitol; Suc, sucrose.

METHODS

Growth of bacteria and preparation of protein extracts. Freeze-dried inocula of the strains listed in Table 1 were cultured in 10 ml of Robertson's cooked meat broth (modified by Watt, 1973) and incubated for 48 h at 37 °C by the standard anaerobic procedure of Collee *et al.* (1972). One ml of this culture was inoculated into 50 ml proteose peptone/yeast extract (PPY) medium (Holbrook *et al.*, 1977). After incubation for 20 h, 3 ml of the culture was removed for purity checks and biochemical testing (Table 1) while the remainder was harvested by centrifugation at 3000 *g* for 30 min and washed three times in phosphate buffered saline (0.15 M-NaCl in 50 mM-Na₂HPO₄/NaH₂PO₄ buffer, pH 7.4). The bacteria were resuspended in 2 ml phosphate buffered saline containing 10 mM-EDTA and transferred to a glass test tube. To remove the outer membranes

and associated molecules, the suspension was incubated for 30 min at 45 °C, then vortex-mixed, treated in an ultrasonic bath (model 6441A; Dawe Instruments, Western Ave, London) for 1 min and again vortex-mixed. After two centrifugations (6000 *g* for 30 min) the supernatant fluid contained outer-membrane vesicles together with released proteins and other molecules. The above conditions were standardized to give optimum release of protein without lysis of the bacterial cytoplasmic membrane. EDTA was removed by dialysis for 4 to 5 h in two successive 1 litre volumes of 0.01 M-Tris/HCl pH 7.4 containing 0.01 % (v/v) 2-mercaptoethanol and the samples were concentrated by dusting the dialysis bags with a fine layer of Sephadex G-100 and leaving at 4 °C for 16 h. The concentrated extracts were freeze-dried and dissolved in SDS-PAGE sample buffer to give a protein concentration of 2 mg ml⁻¹. Sample buffer was 0.0625 M-Tris/HCl pH 6.8 containing 2 % (w/v) SDS, 10 % (v/v) glycerol, 1 % (v/v) 2-mercaptoethanol and 0.001 % (w/v) bromophenol blue. Samples were heated in a boiling water bath for 3 min just prior to application to the gel.

Polyacrylamide gel electrophoresis. This was adapted from the method of Laemmli (1970). Slab gels (170 × 140 mm) of 10 % (w/v) acrylamide with a 10 mm 4 % (w/v) stacking gel were used to run up to 12 samples in a Raven (Haverhill, Suffolk, CB9 7UU) slab gel apparatus. Buffers were as described by Laemmli (1970). Samples (100 µl) of extract containing 200 µg protein were electrophoresed at constant voltage, first at 50 V until the sample had entered the separating gel (approx. 1 h) then at 150 V until the bromophenol blue was near the bottom of the gel (approx. 4 h). Staining was carried out overnight and de-staining was done over 4 h with the solutions described by Poxton & Sutherland (1976).

Protein estimation. Samples of EDTA extracts were assayed for protein using the method of Lowry *et al.* (1951).

Characterization of strains used. All test strains had previously been characterized by the procedures of Duerden *et al.* (1976). Selected biochemical tests, however, were performed on each strain after growth in PPY medium (Table 1). These served as a check on the previous results. They included tests on indole production and the fermentation of rhamnose, trehalose, sucrose and mannitol by the methods described by Duerden *et al.* (1976).

RESULTS AND DISCUSSION

The EDTA-extracted proteins from eight different type species included in the *B. fragilis* group were examined on the same gel (Fig. 1). The patterns of bands were quite different; only a few matching lines were shared and there was no possibility of picking out diagnostic bands typical of the *B. fragilis* group as a whole. However, the individual species within the group showed species-specific patterns.

For 11 strains of the species *B. fragilis* (syn. *B. fragilis* subsp. *fragilis*) there were many matching bands in all of the extracts (Fig. 2), perhaps with the exception of NCTC 8560 (track 2) which did not share many bands with any of the other strains. The double high molecular weight and triple low molecular weight sequences of bands (arrows) appeared to be common to the other 10 strains. There were close similarities between tracks 1 and 5 and between tracks 3, 7, 8, 9, 10 and 11.

Ten strains of *B. vulgatus* (syn. *B. fragilis* subsp. *vulgatus*) gave similar patterns with many matching bands (Fig. 3). The sequence of five or six high molecular weight bands (arrow) appeared to be characteristic of this species. Many other sequences of bands were shared by these strains and an overall similarity of patterns was observed.

Four strains of *B. distasonis* (syn. *B. fragilis* subsp. *distasonis*) were examined with a strain of debated identity (Fig. 4). All four strains of *B. distasonis* gave a strongly staining, high molecular weight band of similar, but not necessarily identical, molecular weight (arrow). The dubious strain (track 2, Fig. 4) had been received as *B. vulgatus* but the results of subsequent biochemical re-testing (Table 1) and the pattern produced on the gel confirmed that it was *B. distasonis*. It was, in fact, identical with strain ATCC 8503 (track 1, Fig. 4). Strains GNAB 27 and GNAB 39 (tracks 4 and 5, Fig. 4) showed almost identical patterns. These were faecal isolates taken several days apart from the same person.

Three indole-positive species of *Bacteroides* which were originally regarded as *B. thetaiotaomicron* (syn. *B. fragilis* subsp. *thetaitaomicron*) are now grouped into at least three separate species: *B. thetaiotaomicron*, *B. eggerthii* and *B. uniformis*. Their patterns on gels (Fig. 5) showed that they share several common polypeptides but there are closer similarities between strains from the same species. The four strains of *B. thetaiotaomicron* (tracks 1 to 4)

Fig. 1. SDS-PAGE of EDTA-extracted cell-surface proteins of *Bacteroides* species on 10% acrylamide slab gels (for details, see Methods). Track 1, *B. fragilis* NCTC 10584; 2, *B. vulgatus* NCTC 10583; 3, *B. distasonis* ATCC 8503; 4, *B. thetaiotaomicron* NCTC 10582; 5, *B. eggerthii* NCTC 11155; 6, *B. ovatus* ATCC 8483; 7, *B. uniformis* ATCC 8492; 8, *B. splanchnicus* NCTC 10825.

Fig. 2. SDS-PAGE of EDTA-extracted cell-surface proteins of strains of *Bacteroides fragilis* on 10% acrylamide slab gels (for details, see Methods). Track 1, NCTC 9344; 2, NCTC 8560; 3, NCTC 10581; 4, NCTC 10584; 5, GNAB 4; 6, GNAB 8; 7, GNAB 49; 8, GNAB 101; 9, GNAB 102; 10, GNAB 104; 11, GNAB 105. Arrows indicate bands characteristic of the species.

Fig. 3. SDS-PAGE of EDTA-extracted cell-surface proteins of strains of *Bacteroides vulgatus* on 10% acrylamide slab gels (for details, see Methods). Track 1, GNAB 25; 2, GNAB 30; 3, GNAB 31; 4, GNAB 34; 5, GNAB 35; 6, GNAB 37; 7, GNAB 38; 8, GNAB 44; 9, GNAB 107; 10, GNAB 120. Arrows indicate bands characteristic of the species.

Fig. 4. SDS-PAGE of EDTA-extracted cell-surface proteins of strains of *Bacteroides distasonis* on 10% acrylamide slab gels (for details, see Methods). Track 1, ATCC 8503; 2, mis-labelled *B. vulgatus*; 3, GNAB 22; 4, GNAB 27; 5, GNAB 39. Arrow indicates a band characteristic of the species.

Fig. 5. SDS-PAGE of EDTA-extracted cell-surface proteins of strains of indole-positive *Bacteroides* species on 10 % acrylamide slab gels (for details, see Methods). *Bacteroides thetaiotaomicron*: track 1, NCTC 10582; 2, GNAB 1; 3, GNAB 2; 4, GNAB 7. *Bacteroides eggerthii*: track 5, NCTC 11155; 6, GNAB 21; 7, GNAB 24; 8, GNAB 41. *Bacteroides uniformis*: track 9, ATCC 8492; 10, VPI 11227; 11, GNAB 18; 12, VPI 11368.

showed many matching bands. Similarly, the four strains of *B. eggerthii* (tracks 5 to 8) closely matched each other. The strains of *B. uniformis* (tracks 9 to 12), however, showed variation: strains ATCC 8492 (track 9) and VPI 11227 (track 10) matched very closely, and strain VPI 11368 (track 12) shared several common polypeptides with these two; however, strain GNAB 18 (track 11) was very different from these although the results from standard biochemical tests showed it to be identical with VPI 11368. Tracks 2 and 4 were unfortunately weakly stained although the amount of protein was the same in all the samples applied to this gel. A duplicate gel, using freshly prepared samples, gave the same result. In the original gel the bands in tracks 2 and 4 could be easily compared with 1 and 3.

This study presents much evidence that similar biotypes of *Bacteroides* possess similar polypeptides in their surface layers. The patterns of *B. fragilis* NCTC 8560 and *B. uniformis* GNAB 18 show, however, that organisms indistinguishable by biochemical tests can differ significantly in their surface chemistry; thus the results of biochemical tests alone can be misleading and should be used with caution. Due to our present lack of knowledge of the cell-surface chemistry and serology of *Bacteroides* we are not in a position to re-classify strain GNAB 18.

The characterization of the *Bacteroides* genus by conventional biochemical techniques is time-consuming and some of the tests are unreliable. SDS-PAGE of surface proteins in conjunction with a few selected biochemical tests offers a rapid, reproducible approach to the demonstration of relatedness between an unknown and a type species. It is hoped that the fine degree of accuracy of this analysis will make it possible to distinguish small differences between strains, so that it might even be possible to distinguish between a newly acquired organism and a resident strain in a patient.

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