

MEDICAL MYCOLOGY

Biochemical characterisation of human isolates of *Blastocystis hominis*

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Summary. SDS-PAGE and iso-enzyme analysis of 11 human isolates of *Blastocystis hominis* revealed at least two variants with different polypeptide patterns and two zymodemes, respectively. This is the first iso-enzyme and the second protein analysis to indicate strain differences in *B. hominis*.

Introduction

Blastocystis hominis is a common protozoan found in the human intestinal tract. It has diversity of shape and size, of which the four distinct forms are recognised: vacular (central body, CB), amoeboid, granular and multiple fission. The last three forms can be derived only from the CB form.¹ The pure amoeboid form seen in rare cases of blastocystosis with very severe symptoms reverts to the typical CB form in culture and *in vivo*, when symptoms subside.² A relationship to *Apicomplexa* and *Sarcodina* has been suggested² but the amoeboid form lacks certain characteristics of these genera. For these reasons Jiang and He¹ suggested a separate classification and assigned the parasite to the subphylum blastocysta. Conversely, Boreham and Stenzel³ felt that *B. hominis* did not fit into existing protozoan classifications and Hollebeke and Mayberry⁴ recommended that *Blastocystis* remain *incerta sedis* until the taxon can be more adequately defined.

Morphologically similar *B. hominis* organisms have been detected in stools of both asymptomatic individuals^{5–6} and patients with diarrhoea^{5–6} and other gastrointestinal symptoms.^{7–10} Such information led Kukoschke *et al.*⁵ to hypothesise that different types of *B. hominis* with the same morphology but with varying pathogenic potential might exist. Kukoschke and Müller¹¹ supported this hypothesis by identifying at least two biochemically and immunologically distinct *B. hominis* strains from man. Moreover, Boreham *et al.*¹² found that human isolates of *B. hominis* constitute at least two distinct groups, differing in their DNA

contents and their protein patterns. The present study was conducted to investigate the extent of the biochemical differences among human isolates of *B. hominis* and to determine if these differences relate to the pathogenic potential of the organism.

Materials and methods

Stool specimens from nine patients with gastrointestinal symptoms and two asymptomatic individuals were examined repeatedly (at least five times) for parasites by the Merthiolate-iodine-formaldehyde-ether concentration method¹³ and by the modified Ziehl Neelsen stain¹⁴ for *Cryptosporidium*. Stools were cultured routinely for pathogenic bacteria and tested serologically for rotavirus. The clinical and laboratory data were used to classify the cases into asymptomatic or symptomatic carriers and patients with acute or chronic gastrointestinal symptoms, according to the definitions of Doyle *et al.*⁶

Culture of *B. hominis*

B. hominis organisms were isolated and grown axenically in a diphasic egg medium under anaerobic conditions as described by Zierdt.¹⁵

Sample preparation

Axenically grown *B. hominis* were pelleted by centrifugation at 4000 *g* for 10 min and the supernate from each isolate was saved as a control. The pellets were washed twice with phosphate-buffered saline (PBS), pH 7.2. Pellets were suspended in a minimum amount of distilled water and sonicated for 40 s with a Sonic 2000 Sonicator (Braun Melsungen, Germany). Protein concentrations of sonicates of 11 *B. hominis*

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isolates were determined with the BioRad Protein Assay kit (BioRad, Richmond, CA, USA).

Each preparation was divided into two portions; one portion was used fresh for iso-enzyme analysis and the other was stored at -20°C until used for protein analysis. The 11 *B. hominis* isolates and their culture supernates were separated electrophoretically by SDS-PAGE slab¹⁶ (acrylamide 12%)¹⁶ and on cellulose acetate membranes for glucose phosphate isomerase (GPI), phosphoglucomutase (PGM), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6-PGD)

and hexokinase (HK) iso-enzyme patterns following the method of Kretzner and Christensen.¹⁷

Results

The 11 *B. hominis*-infected persons comprised two asymptomatic carriers, four patients with acute diarrhoea (including two with salmonella enteritis and one with shigella dysentery) and five patients with chronic gastrointestinal symptoms (including one with giardiasis) (table).

Table. Relationship between the clinical presentation and the biochemical classification of 11 *B. hominis* isolates

Patient no.	Faecal parasites	Bacterial pathogens	Clinical signs and symptoms			Biochemical classification of <i>B. hominis</i>			
			Acute diarrhoea	Chronic diarrhoea	Constipation/diarrhoea	Colic	Flatulence	Protein variant	Zymodeme
1	<i>E. vermicularis</i>							A	Z-I
2	<i>E. coli</i>							B	Z-I
	<i>E. nana</i>								
3	<i>S. mansoni</i>	<i>Shigella</i> sp.	+					B	Z-I
4				+				B	Z-IIa
5				+				B	Z-IIa
6	<i>G. lamblia</i>							B	Z-IIa
7					+		+	B	Z-IIa
8							+	B	Z-IIa
9		<i>Salmonella</i> sp.	+					B	Z-IIa
10		<i>Salmonella</i> sp.	+					B	Z-IIb
11			+					B	Z-IIb

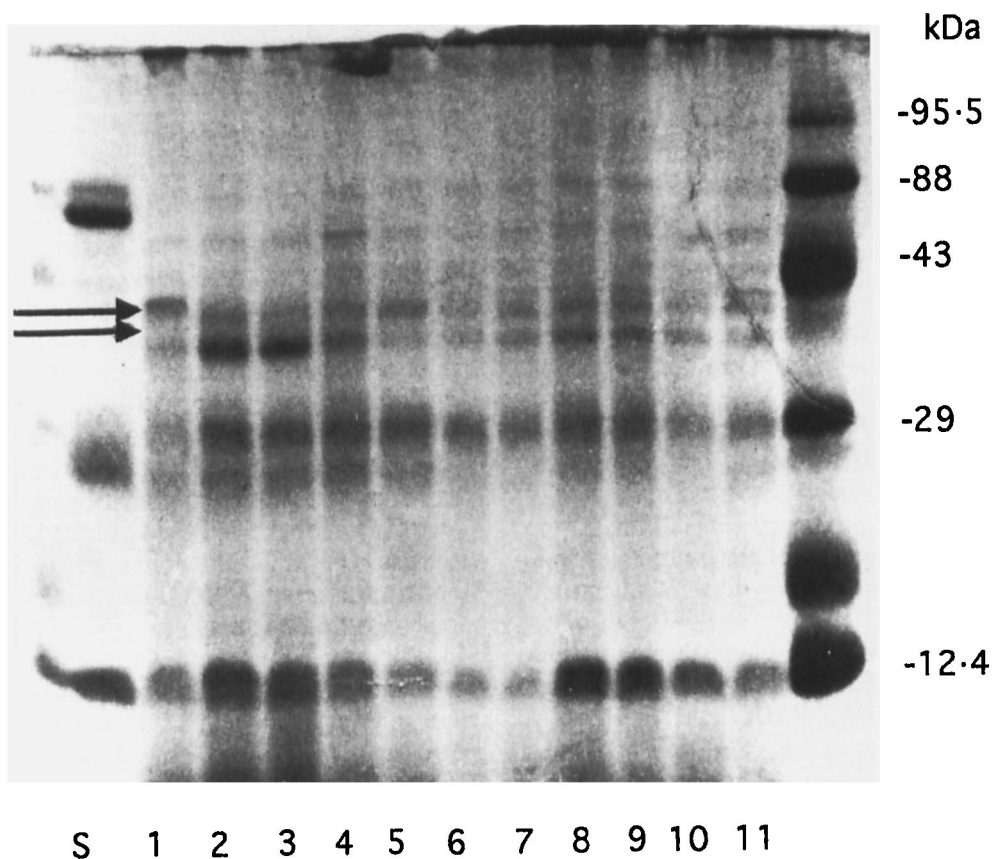


Fig. 1. SDS-PAGE (12% acrylamide) separation of total protein from axenic culture supernate (S) *B. hominis* isolates (1–11), and mol. wt markers. The arrows indicate significant differences between isolate no. 1 and the rest of the isolates.

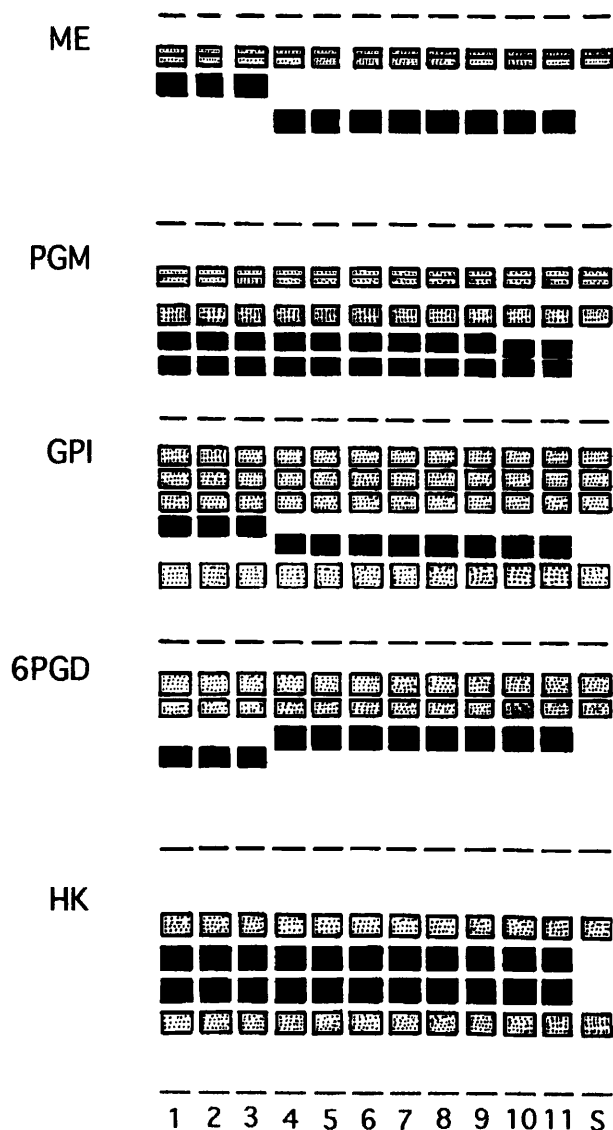


Fig. 2. Diagrammatic representation of malic enzyme (ME); phosphoglucosmutase (PGM); glucose phosphate isomerase (GPI); 6-phosphogluconate dehydrogenase (6-PGD); hexokinase (HK) after electrophoresis on cellulose acetate membranes. Numbers 1-11 indicate *B. hominis* isolate number, S, culture supernate.

The SDS-PAGE protein banding patterns of *B. hominis* isolates were characterised by common major protein bands of 12- > 95 kDa. The banding patterns were identical in 10 of the 11 isolates. The variant pattern in one isolate was characterised by the presence of an additional 40-kDa band and the absence of a 35-kDa band (fig. 1). The protein patterns of the culture supernate (used as controls) of the 11 isolates were identical but were distinct from those of *B. hominis* organisms.

Iso-enzymes of *B. hominis* isolates resolved into a single dense band in ME, GPI and 6-PGD and double dense bands in both PGM and HK (fig. 2). Analysis of the iso-enzyme patterns of the 11 isolates generated two profiles which were classified as two zymodemes, designated as Z-I and Z-II (table). Z-I was represented by *B. hominis* from two asymptomatic carriers and one symptomatic carrier who also had shigellosis. Z-II was represented by *B. hominis* from eight symptomatic

patients. It was characterised by a faster migrating band in 6-PGD and a slower migrating band in ME and GPI, compared with those of the three isolates designated as Z-I. *B. hominis* from the two symptomatic patients with acute diarrhoea belonging to Z-II had a slower migrating band in one of the two PGM bands than the corresponding ones in the other nine isolates (fig. 2). The banding patterns of the five enzymes were identical in the culture supernates of the 11 isolates but were distinct from those of *B. hominis* isolates.

Discussion

In the present study, the protein profiles of the 11 *B. hominis* isolates clearly demonstrated the existence of two variants, which were differentiated by the 35- and 40-kDa bands. One of the two variants was represented by *B. hominis* from nine symptomatic patients and one asymptomatic individual. These results differ from those of Kukosche and Müller⁷ who also found variants that differed in the 40-, 45- and 50-kDa bands; one variant was isolated from two symptomatic patients and the other from an asymptomatic person and a patient suffering from diarrhoea. Furthermore, Boreham *et al.*¹² examined 10 *B. hominis* isolates and demonstrated two distinct groups differing in their protein and DNA contents, with significant differences between their genomes.

Iso-enzyme analysis, conducted for the first time on *B. hominis*, generated two zymodemes (Z-I and Z-II). Z-I included three isolates from the two asymptomatic patients and one patient with acute diarrhoea attributable to shigellosis. The other zymodeme (Z-II) included eight isolates, all from symptomatic patients (although two patients had proven salmonellosis). Furthermore, the latter zymodeme included two variants that were identical in their patterns of the five enzymes but differed in the migration of one of the two PGM bands. One of these variants (Z-IIa) included isolates from five patients with chronic gastrointestinal symptoms and one patient with salmonellosis. The other variant (Z-IIb) included two isolates associated with acute diarrhoea (table). The iso-enzyme analysis provided a better classification than the protein analysis of *B. hominis* from symptomatic and asymptomatic individuals. The five enzymes used in this study have been used previously to differentiate pathogenic from non-pathogenic strains of *Entamoeba histolytica*¹⁸ and vahlkamfiid amoebae.¹⁹

In the current study, mixed infections made it difficult to ascertain whether the clinical symptoms were due to *B. hominis* or to the other associated pathogens. Therefore, further studies are encouraged to determine the extent of differences among the iso-enzyme and DNA hybridisation patterns of more well documented *B. hominis* isolates from symptomatic and asymptomatic persons and to find out if these differences relate to the clinical presentations of the

hosts reflecting the pathogenic potential of the organism.

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