# Study of Protease Enzyme Activity of *Blastocystis* isolates from Gastrointestinal Symptomatic and Asymptomatic Subjects

**Thesis**

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*To my dear husband*

*and my kids*

*To my dear mother and family*

*To the soul of my father*

# ABSTRACT

The present study was carried out on 62 subjects positive for *Blastocystis* and presenting with gastrointestinal symptoms or asymptomatic. The symptomatic group (cases,**GI**) included 42 cases (67.7%) while the asymptomatic (control,**GII)** group included 20 subjects (32.2%). The study aimed to investigate the protease activity and protein profiles of *Blastocystis* isolates to determine if differences could be related to the patient symptomatic status. Using SDS-PAGE analysis, the protein profiles of *Blastocystis* isolates showed 22 protein bands, ranged from 12 to 200 kDa. The study showed non statistical significant differences between symptomatic and asymptomatic groups at different molecular weights.

Using gelatin SDS-PAGE analysis, the protease profiles of *Blastocystis* isolates showed 14 protease bands of both high and low molecular weights with significant differences between symptomatic and asymptomatic groups at 35, 60 and 140 kDa MW bands.

Using Azocasein assay, *Blastocystis* isolates from symptomatic cases show quantitatively higher protease activity than asymptomatic cases but without significant difference between the groups.

**Key words:** *Blastocystis,* In vitro culture, Morphological forms, Protein profile, Protease, SDS-PAGE, Gelatin SDS-PAGE, Azocasein assay

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# List of Abbreviations

| | |
|---|---|
| APS | Ammonium persulfate |
| BSA | Bovine serum albumin |
| °C | Degree celcius |
| cm | Centimeter |
| CPs | Cysteine proteases |
| DNA | Deoxyribonucleic acid |
| DTT | Dithiothretol |
| EDTA | Ethylene diamine tetraacetate |
| EF | Elongation factor |
| ELISA | Enyme Linked Immunosorbant assay |
| g | Gravitional acceleration |
| g | Gram |
| GIT | Gastrointestinal |
| h | Hour |
| HCL | Hydrochloric acid |
| IBS | Irritable bowl syndrome |
| Ig | Immunoglobulin |
| IL | Interleukin |
| IFA | Indirect fluorescent antibody |
| KCl | Potassium chloride |
| kDa | Kilo Dalton |
| $KH_2PO_4$ | Potassium dihydro phosphate |
| min | Minute |
| ml | milliliter |
| MLOs | mitochondria- like organelles |
| mm | Millimeter |
| mM | Millimole |
| MW | Molecular weight |
| n | Number |
| NA | Not available |
| NaCl | Sodium chloride |
| $Na_2HPO_4$ | Sodium dihydro phosphate |

| | |
|---|---|
| NaOH | Sodium hydroxide |
| nm | Nanometer |
| no | Number |
| OD | Optical density |
| PARs | Protease-activated receptors |
| PBS | Phosphate buffer saline |
| PCR | Polymerase chain reaction |
| PH | Hydrogen Potential |
| *P* value | Probability |
| *q PCR* | Quantitative Polymerase chain reaction |
| r RNA | ribosomal ribonucleic acid |
| SD | Standard deviation |
| SDS-PAGE | Sodium dodecyle sulphate poly acrylamide gel electrophoresis |
| spp. | Species |
| SSU-rRNA | Small subunit ribosomal ribonucleic acid |
| ST | Subtype |
| TCA | Trichloroacetic acid |
| TEM | Transmission electron microscopy |
| TEMED | N, N, N, N tetramethylethylenediamine |
| Tjs | Tight junctions |
| UVR | Ultraviolet radiation |
| V | volt |
| µ | micron |
| µl | microliter |
| µm | micrometer |

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# INTRODUCTION

*Blastocystis* spp. is an anaerobic parasite that inhabits the intestinal tract of humans and a wide range of animals **(Tan, 2004 and Stensvold *et al.*, 2009).** This emerging parasite with a worldwide distribution is often identified as the most common eukaryotic organism reported in human faecal samples, and its prevalence has shown a dramatic increase in recent years **(Rayan *et al.*, 2007 and Tan 2008).**

*Blastocystis* prevalence is higher in less developed countries. The difference in prevalence between developed and developing countries can be explained by poor hygiene practices and consumption of contaminated food or water since the faecal-oral route is considered to be the main mode of transmission of this parasite **(Li *et al.*, 2007; Leelayoova *et al.*, 2008 and Nagel *et al.*, 2012).**

A higher risk of *Blastocystis* infection has been found in humans with close animal contact reinforcing the zoonotic potential of this parasite **(Yan *et al.*, 2007 and Yoshikawa *et al.*, 2009).**

Regarding the pathogenic potential of *Blastocystis*, it has been widely debated in the literature during the last two decades because the organism can be found in both symptomatic and asymptomatic patients. However, numerous recent in vivo and in vitro studies strongly suggest that this organism is a pathogen **(Kaya *et al.*, 2007; Boorom *et al.*, 2008 and Poirier *et al.*, 2012).**

Major symptoms associated with *Blastocystis* infection include diarrhoea, abdominal pain, fatigue, constipation, flatulence, and skin rash but this parasite may also play a significant role in several chronic gastrointestinal illnesses such as irritable bowel syndrome **(Yakoob *et al.*, 2004; Jones *et al.*, 2009 and Clark *et al.*, 2013).**

*Blastocystis* is a polymorphic organism with four major forms described in stools and in vitro cultures: vacuolar, granular, amoeboid, and cyst forms. The amoeboid form has been suggested to play a role in

pathogenesis **(Stenzel and Boreham 1996; Zhang *et al.*, 2007 and Suresh *et al.*, 2009).** The water-resistant infective cyst possibly represents the transmissible form of the parasite **(Suresh *et al.* 2005 and Souppart *et al.*, 2010).**

Besides heterogeneity in morphology, *Blastocystis* is genetically and antigenically diverse, both within and among geographical regions, suggesting that several strains or species of this parasite exist **(Tan, 2008).** In spite of the extensive genetic variability present in *Blastocystis* from humans and animals, a definite correlation between the genotypes and pathogenicity has not yet been confirmed **(Noël *et al.*, 2005; Souppart *et al*., 2009 and Stark *et al.*, 2010**).

Phenotypic differences between *Blastocystis* isolates have been shown through ultrastructural study, isoenzyme patterns, secretion of proteases, protein profiling and sero-groups **(Mansour *et al.*, 1995; Santos and Rivera, 2009; Rajamanikam and Govind, 2013 and Ragavan *et al.*, 2014).**

Cysteine proteases are reported to play important roles in development, differentiation, and pathogenicity of protozoan parasites **(Puthia *et al.*, 2005). Sio *et al.* (2006)** proved that *Blastocystis* is able to produce a cysteine protease that breaks up IgA antibody, which allows *Blastocystis* survival and colonization in the human gut.
Recent studies investigating the pathogenic potential of *Blastocystis* in humans have focused on genotypic analysis without providing phenotypic information on the isolates studied **(Kaneda *et al.*, 2001; Yoshikawa *et al.*, 2004a and Stensvold *et al.*, 2007a).**

Based on these data, the present work aimed to investigate the protease activity of *Blastocystis* isolates obtained from symptomatic and asymptomatic individuals in order to determine whether such an approach can assist in determining potential pathogenicity. The study also included analysis of the protein profiles of *Blastocystis* isolates so as to assess the degree of heterogeneity of this organism.

# AIM OF WORK

1- Investigation of the protease activity of *Blastocystis* isolates obtained from stool samples of symptomatic and asymptomatic individuals using gelatin SDS-PAGE and azocasein assay.

2- Correlation between protease activity, symptomatology and morphological forms of *Blastocystis* isolates.

3- Analysis of the protein profiles of *Blastocystis* isolates using SDS-PAGE and its correlation with the symptomatic status.

# Taxonomy and Historical Background

Kingdom: Chromista
Subkingdom: Chromobiota
Phylum: Stramenopiles
Subphylum: Opalinata
Class: Blastocystea
Order: Blastocystida
Family: Blastocystidae
Genus: *Blastocystis* **(Arisue *et al*., 2002)**

*Blastocystis* is a single-celled, genetically heterogeneous protist, phylogenetically placed within the Stramenopiles **(Arisue *et al*., 2002).**

Various observations were made over years to arrive at the present taxonomical status of *Blastocystis* and this is probably due to the possession of unique phenotypic characters intermediary to different organisms **(Parija and Jeremiah, 2013).**

*Blastocystis hominis* was first described in 1912 as a yeast **(Brumpt 1912).** Subsequent physiological and electron microscopic studies indicated that it is more likely belonging to the protozoa **.** The parasite protistan features include: the presence of one or more nuclei, smooth and rough endoplasmic reticulum, Golgi complex and mitochondrion-like organelles. Moreover, *Blastocystis* failed to grow on fungal media and was not killed by antifungal drugs and some antiprotozoal drugs showed activity against the parasite **(Zierdt, 1976 and 1991)**. **Zierdt (1991)** classified the organism initially as a sporozoan based on morphology,