



Brief Report

# Stool Microbiota Diversity Analysis of *Blastocystis*-Positive and *Blastocystis*-Negative Individuals

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**Abstract:** *Blastocystis* is a unicellular eukaryote found in the gastrointestinal tract of both human and other animal hosts. The clinical significance of colonic *Blastocystis* colonization remains obscure. In this study, we used metabarcoding and bioinformatics analyses to identify differences in stool microbiota diversity between *Blastocystis*-positive and *Blastocystis*-negative individuals (n = 1285). Alpha diversity was significantly higher in *Blastocystis* carriers. At phylum level, Firmicutes and Bacteroidetes were enriched in carriers, while Proteobacteria were enriched in non-carriers. The genera *Prevotella*, *Faecalibacterium*, *Flavonifracter*, *Clostridium*, *Succinivibrio*, and *Oscillibacter* were enriched in carriers, whereas *Escherichia*, *Bacteroides*, *Klebsiella*, and *Pseudomonas* were enriched in non-carriers. No difference in beta diversity was observed. Individuals with *Blastocystis*-positive stools appear to have gut microbiomes associated with eubiosis unlike those with *Blastocystis*-negative stools, whose gut microbiomes are similar to those associated with dysbiosis. The role of *Blastocystis* as an indicator organism and potential modulator of the gut microbiota warrants further scrutiny.

**Keywords:** gut; parasite; *Blastocystis*; microbiome; microbiota; protist; ecology; eubiosis

## 1. Introduction

*Blastocystis* is an intestinal parasitic protist that has puzzled clinical microbiologists and gastroenterologists for decades. It is estimated that more than one billion people may be colonized by this organism [1]; in Denmark, we estimate that about 35% of the adult population are carriers [2]. Studies serving to unravel the role of *Blastocystis* in gastrointestinal health and disease have identified fecal microbiota diversity differences between individuals colonized by *Blastocystis* and those who are not [3–7]. However, the studies published so far have typically been limited by relatively small sample sizes, yielding conflicting results. In this study, the microbiota data from more than one thousand fecal samples from *Blastocystis*-positive (carriers) and *Blastocystis*-negative (non-carriers) individuals from various geographical regions were studied. We submitted all data obtained by

metabarcoding to bioinformatics analysis to identify potential differences in fecal microbiota diversity. The results possibly suggest a role for *Blastocystis* as an indicator organism and a potential driver of gastrointestinal health and disease.

## 2. Materials and Methods

Sequence read data for a total of 1285 fecal DNAs generated by our in-house metabarcoding assay [8–10] for various independent research projects and routine diagnostic purposes were collated and analyzed using our in-house bioinformatics data pipeline previously described [8,9,11]. The fecal DNAs were from populations sampled in Algeria, Egypt, Turkey, United Kingdom, and Denmark. Data were fully anonymized; hence, no clinical or demographic data were available for study.

A sample was classified as positive for *Blastocystis*, if >1.5% of the total read count within the sample mapped to *Blastocystis* at genus level. We chose this positivity cut-off to avoid false positives resulting from sequencing contamination (carryover of ID tags) and because the assay has a preferential bias toward amplifying DNA from *Blastocystis* over other eukaryotic organisms.

We carried out analyses for alpha (Shannon's diversity index and richness) and beta (principal coordinate analysis (PCoA) of Bray–Curtis distances) diversity as well as linear discriminant analysis effect size (LEfSe) to identify and visualize taxonomic differences in microbiota signatures between carriers and non-carriers. Probability ( $p$ ) values < 0.05 were considered to indicate statistically significant differences.

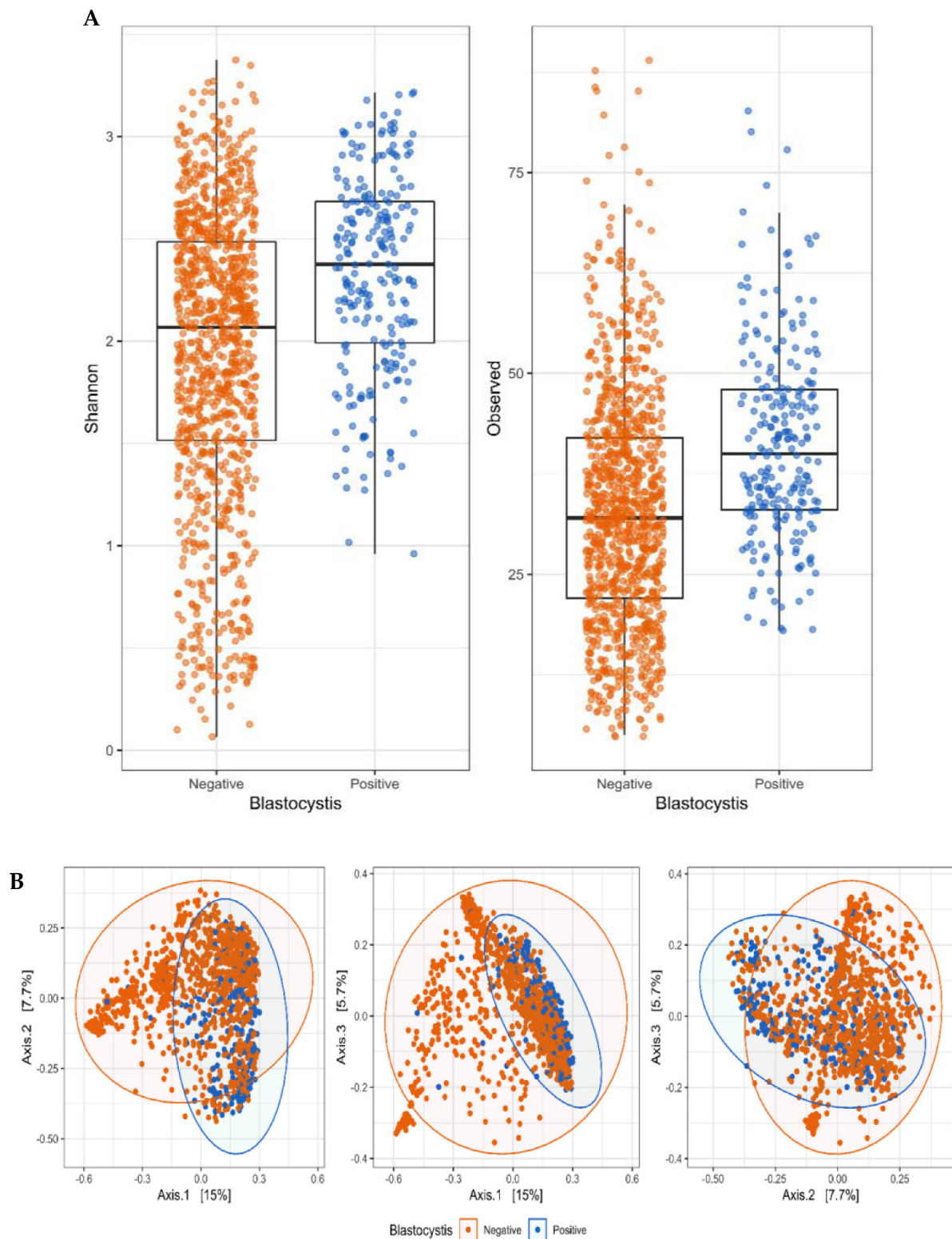
## 3. Results

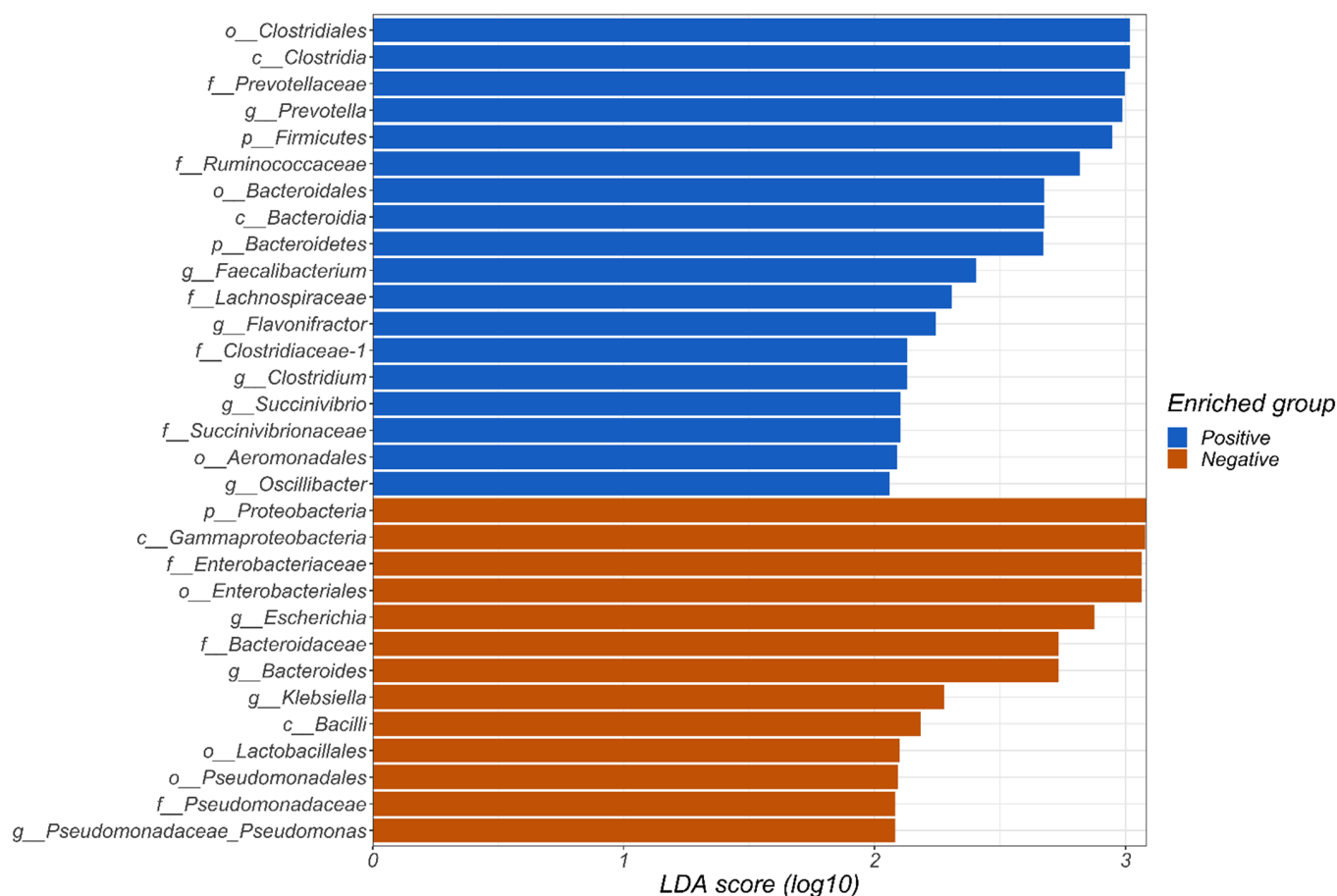
Of the 1285 samples, for which data were available for this study, 235 (18.3%) were positive and 1050 (71.7%) were negative for *Blastocystis*.

Alpha diversity was significantly higher in the *Blastocystis*-positive samples than in the *Blastocystis*-negative samples for both Shannon diversity index ( $p < 0.0001$ ) and observed richness ( $p < 0.0001$ ) (Figure 1A).

With regard to beta diversity (Bray–Curtis; Figure 1B), no clear separation of the groups could be observed in the PCoA plot, suggesting no difference in beta diversity; however, there was a significant statistical difference between the two groups ( $p = 0.001$ ). The statistically significant result may be explained by within-group variation, as the multivariate variation differed significantly ( $p < 0.0001$ ) between groups. The fecal microbiota of carriers appeared to be more similar than that of non-carriers (Figure 1B).

At phylum level, LEfSe analysis revealed that Firmicutes and Bacteroidetes were enriched in carriers, while Proteobacteria were enriched in non-carriers (Figure 2). At genus level, *Prevotella*, *Faecalibacterium*, *Flavonifracter*, *Clostridium*, *Succinivibrio*, and *Oscilibacter* among others were enriched in carriers, while *Escherichia*, *Bacteroides*, *Klebsiella*, and *Pseudomonas* were enriched in non-carriers, among others (Figure 2).





**Figure 2.** Linear discriminant analysis effect size (LEfSe) analysis of the microbiota in *Blastocystis*-positive and *Blastocystis*-negative fecal samples showing the bacterial taxa that were enriched in carriers and non-carriers, respectively. Taxa illustrated by blue bars are those enriched in *Blastocystis*-positive samples, whereas taxa illustrated by orange bars are those enriched in *Blastocystis*-negative samples. The small letters provided at the beginning of each taxon indicate the taxonomic tier (hence, ‘g’ is genus, ‘f’ is family, ‘c’ is class, etc.).

#### 4. Discussion

Higher gut microbiome diversity appears to be conducive to gut health [12]. The higher bacterial diversity observed in individuals colonized by *Blastocystis* may indicate that these individuals typically have a healthier gut microbiota than those who do not carry the organism. In the healthy gut, metabolites such as short-chain fatty acids are developed by fermentation by anaerobic bacteria and possibly also by *Blastocystis* [13]. The metabolism of butyrate may favor a hypoxic intestinal lumen, which again favors colonization by *Blastocystis*, while disfavoring facultative anaerobic bacteria [13].

The enrichment in the families Prevotellaceae, Ruminococcaceae, and Clostridiaceae 1 in carriers supports our previous findings [3,4]. We have previously shown that *Blastocystis* is common in individuals with *Prevotella*- and *Ruminococcus*-driven enterotypes, whereas those with a *Bacteroides*-driven enterotype are rarely colonized [4].

In a metanalysis of 12 large metagenomics data sets, Beghini et al. [14] identified a generally higher abundance of the Bacteroidetes phylum in *Blastocystis*-negative samples, while identifying a strong enrichment of Firmicutes and Clostridiales in *Blastocystis* carriers.

In the present study, the Proteobacteria phylum was enriched in non-carriers as compared with carriers. Interestingly, another study suggested Proteobacteria as a potential diagnostic signature of dysbiosis and risk of disease [15]. Beghini et al. did not specifically report a consistent association between enrichment of Proteobacteria and absence of *Blastocystis*; however, based on the LEfSe analysis included in that study, both Gammapro-

teobacteria and Proteobacteria were 1000–10,000 times enriched in *Blastocystis*-negative samples [14], a finding that is in agreement with the observations of the present study (Figure 2).

The present study was limited by the fact that no data on the health status of the tested individuals were available, which precluded investigation of the relationship between the presence of *Blastocystis* and specific factors associated with health and disease. Future studies should analyze microbiota data of carriers and non-carriers in the context of demographic and clinical data, such as functional and inflammatory bowel diseases. Nevertheless, given the findings presented here, those presented previously by our group [3,4,9], and those presented by independent research teams [5,6], it appears that *Blastocystis* could be considered a biomarker of intestinal eubiosis. Moreover, a recent study identified *Blastocystis* as an indicator of favorable postprandial glucose metabolism [16], and Beghini et al. recently identified a link between normal body mass index and *Blastocystis* colonization [14]. Further studies should couple bioinformatics analyses with clinical and demographic data and investigate whether *Blastocystis* is merely an indicator organism or also an active driver of gut microbiota diversity and host metabolism.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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