



IN VITRO EVALUATION OF THE EFFECTS OF TWO COMMON PROBIOTICS (*Saccharomyces boulardii* AND *Bacillus clausii*) ON *Blastocystis*

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Abstract: The role of *Blastocystis* in gut health is debated, with most studies recognizing it as a member of a balanced gut microbiota. We investigated the in vitro effects of two common probiotics (*Saccharomyces boulardii* and *Bacillus clausii*) on *Blastocystis*. A *Blastocystis* positive stool sample was cultured in Jones medium, and the subtype identified by sequencing the 18S rRNA barcode region. Effects of probiotics (4×10^7 , 4×10^8 , and 4×10^9 CFU/mL) on *Blastocystis* viability and growth were tested with the Trypan Blue dye exclusion assay over 12 - 96 h. Metronidazole (100 µg/mL) was used as the reference drug. *Blastocystis* isolate from a patient with abdominal pain was cultivated and the subtype was identified as ST3 after sequence analysis. In vitro co-incubation with *S. boulardii* completely inhibited *Blastocystis* growth at all doses ($P < 0.05$), while *B. clausii* showed no significant effect compared to control. Morphologically degenerated *Blastocystis* cells were observed in the metronidazole-treated group in the same period. Our findings support that *S. boulardii* has the potential to effectively inhibit *Blastocystis* growth in vitro and suggest that its promising efficacy may be strain-dependent or species-specific. Further biochemical and molecular studies are required to clarify mechanisms underlying the therapeutic potential of *S. boulardii* to manage *Blastocystis* infections.

Keywords: *Blastocystis*, *Saccharomyces boulardii*, *Bacillus clausii*, Probiotics, in vitro

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Received: February 26, 2026

Accepted: May 08, 2026

Published: May 15, 2026

Cite as: Demir, Z. E., Damar, B. N., Doğan, A. S., & Malatyali, E. (2026). In vitro evaluation of the effects of two common probiotics (*Saccharomyces boulardii* and *Bacillus clausii*) on *Blastocystis*. *Black Sea Journal of Health Science*, 9(3), 104-110.

1. Introduction

Blastocystis is a eukaryotic microorganism with a wide global distribution that infects humans and animals (Yoshikawa and Iwamasa, 2016). The life cycle comprises several major morphological forms, including vacuolar, granular, amoeboid and cystic stages. The primary mode of transmission is fecal-oral. It is a genetically diverse microorganism that relies on high polymorphism in the small subunit ribosomal RNA gene (SSU rRNA). To date, 48 *Blastocystis* subtypes (ST1-ST48) have been identified, with ST3 being the most prevalent subtype in humans (Sejnovhova et al., 2024). The pathogenicity of *Blastocystis* remains uncertain, as it may act as either a commensal or a pathogen depending on host interactions, virulence factors, and subtypes (Pawelec-Peciak et al., 2025). *Blastocystis* infection is mostly asymptomatic; however, it has been associated with gastrointestinal symptoms, such as abdominal pain, diarrhea, distension, and urticaria (Tan, 2008). Immunosuppressed individuals may have increased susceptibility to these clinical manifestations. A growing body of literature has reported associations between *Blastocystis* and inflammatory or autoimmune disorders,

including irritable bowel syndrome (IBS), ulcerative colitis, colorectal cancer, and Hashimoto's disease (Pawelec-Peciak et al., 2025). The human gut microbiome is a dynamic ecosystem comprising bacteria, archaea, viruses, and eukaryotic microorganisms. Studies on *Blastocystis* and microbiota indicate that their presence is linked to microbial balance and diversity. This suggests that *Blastocystis* may function as a commensal ecosystem component rather than a pathogen, clinical symptoms may emerge if this balance is disrupted (Aykur et al., 2024).

Agents such as metronidazole (MZ), trimethoprim-sulfamethoxazole (TMP-SMX), paromomycin, nitazoxanide (NTZ), ornidazole, secnidazole, and tinidazole (TIN) are used to treat *Blastocystis* infections in humans. Although MZ is the most used agent, recent studies have reported the presence of resistant isolates. The rising MZ resistance suggests that antiparasitic drugs alone may be inadequate and highlights the necessity for new therapeutic strategies for *Blastocystis* infection (Roberts et al., 2015; Uluşan Bağcı and Aral Akarsu, 2025). Probiotics are live microorganisms that, when administered in adequate amounts, confer a health profit



on the host. The primary function of probiotics is to modulate the gut microbiota, supporting the integrity of the host's intestinal barrier and immune system (Mazziotta et al., 2023). Probiotics are used to manage various parasitic diseases, including giardiasis, coccidiosis, amoebiasis, cryptosporidiosis, and leishmaniasis (Mandal et al., 2024). Among probiotic microorganisms, some are frequently preferred in therapy because of their proven clinical efficacy. Although *Lactobacillus* and *Bifidobacterium* are the most extensively studied, other strains with distinct biological advantages have also been reported. *Bacillus clausii* (*Alkalihalobacillus clausii*) is a probiotic bacterium that has been widely used for decades because of its beneficial effects on health. Its capacity to form heat, acid, and salt resistant spores provides safe passage through the gastrointestinal tract without loss of viability (Ghelardi et al., 2022).

Saccharomyces boulardii is a probiotic yeast widely used to manage bacterial diarrhea and maintain intestinal flora balance (Alsalemi et al., 2025). It has special features, such as the ability to survive at different pH levels, resist antibiotics, and maintain stable populations. These features make it superior to most bacterial probiotics (Tomičić et al., 2024). This probiotic reduced *Giardia*-induced mucosal damage and oxidative stress caused by both the parasite and metronidazole (MTZ) (Ribeiro, 2018).

Although the beneficial effects of probiotics on gastrointestinal health have been widely reported, their effects on *Blastocystis* have been poorly investigated (Lepczyńska and Dzika, 2019; Aykur et al., 2024).

Although some studies have investigated the application of *S. boulardii* in clinical settings, no in vitro studies have been conducted. A previous study on children reported that the cure rate of *S. boulardii* was comparable to that of metronidazole, challenging the guidelines (Dinleyici et al., 2011). In addition, a gradual reduction in *Blastocystis* number in the mice fecal samples was observed after treatment with *S. boulardii* (Abd AL-Khaliq et al., 2015). This probiotic also improved histological features and reduced cytokine and iNOS levels in the colonic mucosa of rats (Méabed et al., 2019). No data is available in the literature, either in vivo or in vitro, on the effect of *B. clausii* on *Blastocystis*. This study aimed to evaluate the in vitro effects

of *S. boulardii* and *B. clausii* on *Blastocystis* viability and growth.

2. Materials and Methods

2.1. *Blastocystis* Isolate and Cultivation

Routine stool samples obtained from Aydin Adnan Menderes University Faculty of Medicine were used in this study. A *Blastocystis* positive stool sample identified by direct microscopic examination was inoculated into Jones medium. Rice-sized fresh fecal samples were transferred into the medium.

The stock solution of Jones medium was prepared as

follows: 1.15% $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 0.9% KH_2PO_4 , and 0.9% NaCl were mixed in volumes of 93.8, 31.3, and 562.5 mL, respectively. Following the addition of 0.69 g yeast extract and adjustment of the pH to 7.1. The medium was autoclaved at 121 °C for 20 min. The solution was cooled to room temperature and supplemented with 10% fetal calf serum (FCS). The medium was dispensed in 3 mL aliquots into 5 mL glass tubes. The cultures were incubated at 35 °C in a standard bacteriological incubator. Subcultures were performed by addition of 0.5 mL *Blastocystis* positive medium to 3 mL of fresh medium. Parasite growth was checked after 48 h, and the *Blastocystis* suspension was used for viability experiments.

2.2. Detection of *Blastocystis* Subtype

To detect the subtype of the cultured *Blastocystis* isolate, genomic DNA was extracted from 200 mg of stool sample using a commercial kit (Magen, Guangzhou, China), according to the manufacturer's instructions. The *Blastocystis* barcode region was amplified by polymerase chain reaction (PCR) using the RD5 (Forward) (5'-ATC TGG TTG ATC CTG CCA GT-3') and BhRDr (Reverse) (5'-GAG CTT TTT AAC TGC AAC AAC G-3') primers, as described by Scicluna et al. (2006). In PCR, the ADUBI201 isolate (GenBank accession no. KU361300) was used as the positive control. The resulting amplicons were sequenced at a commercial facility (Medsantek, Istanbul, Türkiye) using an Applied Biosystems 377 DNA sequencer. Subtype identification was performed based on exact matches in the *Blastocystis* 18S rRNA database (<http://pubmlst.org/Blastocystis>).

2.3. The Effect of Probiotics on *Blastocystis*

Two commercially available probiotics, *S. boulardii* (Rawflor) and *B. clausii* (Enterogermina), were used in the experiments. Probiotic suspensions were prepared as stock solutions and added to *Blastocystis* cultures at final concentrations of 4×10^7 , 4×10^8 , and 4×10^9 CFU/mL. The initial *Blastocystis* density in the cultures was 5–10 cells per 40× microscopic field. *Blastocystis* counts and viability were determined using the Trypan blue dye exclusion method. For this purpose, 10 µL of the culture sample was mixed with an equal volume of Trypan Blue solution, and cell counting was performed under a light microscope at 10× or 40× magnification. The control group did not include any substance and incubated like study groups. All measurements were performed in triplicates. The data are presented as the mean number of *Blastocystis* at 12, 24, 48, 72, and 96 h. Culture tubes showing no detectable growth were subcultured in fresh media for verification. Metronidazole (Flagyl, 500 mg) was tested as a reference drug at 100 µg/mL (Aykur et al., 2020).

2.4. Statistical Analysis

Statistical analyses were performed using Prism version 8.0.2 for Windows (GraphPad Software, USA). Normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated with Levene's test. As the data did not meet the assumption of

normality ($P < 0.05$), the groups were compared with the Kruskal–Wallis test, and pairwise comparisons were performed using Dunn’s test and Bonferroni correction. Data were presented as mean $\pm$ standard deviation (SD). Statistical significance level was set at $P < 0.05$ (Genç and Soysal, 2018; Önder, 2018).

3. Results

Blastocystis was isolated from the fecal sample of a male patient in the Gastroenterology Department who was suffering from constipation and abdominal pain. The subtype of the *Blastocystis* isolate was identified as ST3, allele 34, based on an exact match with the *Blastocystis* database. The partial SSU rRNA sequence was submitted to GenBank under accession number PX458647.

Probiotics demonstrated time- and dose-dependent effects on *Blastocystis* proliferation. In the *S. boulardii*-treated group, *Blastocystis* was non-viable and not visible except after 12 h of incubation with 4×10^7 and 4×10^8 CFU/mL (Figure 1). In contrast, the *B. clausii*-treated group consistently showed viable and visible *Blastocystis*

at all doses (Figure 2). All *Blastocystis* cells in the control group remained alive throughout the 72-hour study period (Figure 3). At the lowest probiotic concentration (4×10^7 CFU/mL), pairwise comparisons revealed a significant difference between the *S. boulardii* and control groups at all-time points ($P < 0.05$), whereas no significant differences were observed between the control and *B. clausii* groups ($P > 0.05$). In addition, for the other concentrations and time points, *Blastocystis* counts for *S. boulardii* were significantly lower than those of *B. clausii* and the control group ($P < 0.05$). No significant difference was observed between *B. clausii* and control at same time points (Table 1 and Figure 4). Metronidazole efficiently reduced the number of viable *Blastocystis* cells between 12 and 72 h. However, both trypan blue–stained non-viable cells and unstained but morphologically degenerated cells were observed (Figure 5). Morphological degeneration was mostly characterized by the loss of the typical vacuolar appearance, cytoplasmic contraction, and membrane disruptions.

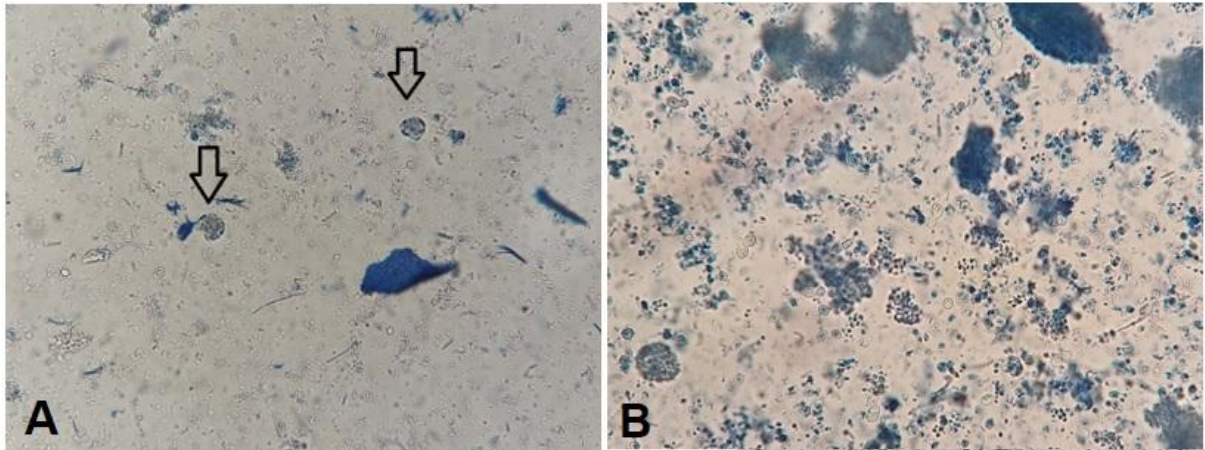


Figure 1. *Blastocystis* viability after treatment with *S. boulardii* at 40 $\times$ magnification: (A) after 24 h incubation with 4×10^7 CFU/mL *S. boulardii* showing two trypan blue–stained non-viable *Blastocystis* cells (black arrows), (B) after 24 h incubation with 4×10^9 CFU/mL *S. boulardii* showing complete clearance of *Blastocystis* cells.

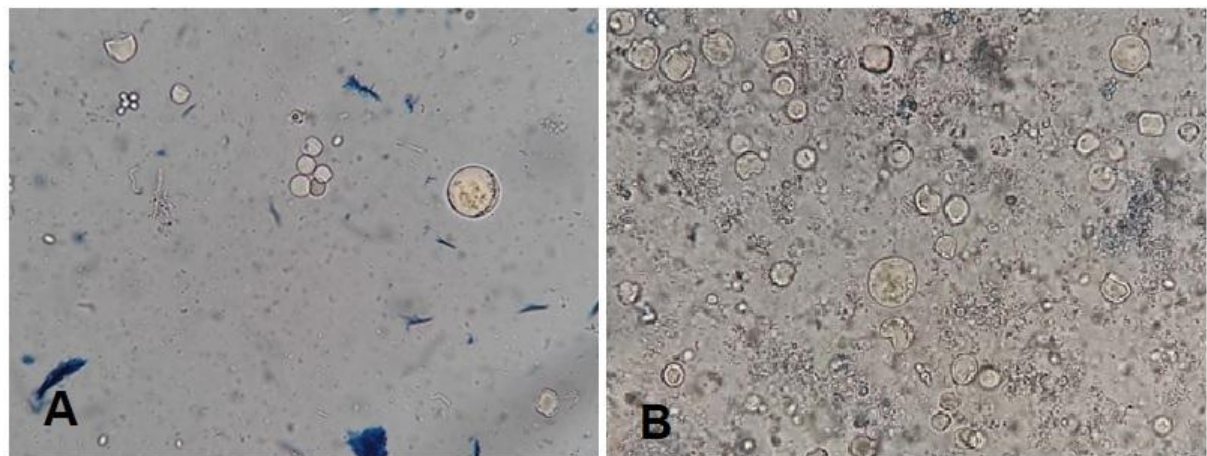


Figure 2. *Blastocystis* viability after treatment with *B. clausii* at 40 $\times$ magnification, stained with trypan blue: (A) after 48 h incubation with 4×10^7 CFU/mL *B. clausii* showing live *Blastocystis* cells, (B) after 96 h incubation with 4×10^8 CFU/mL *B. clausii* showing numerous viable *Blastocystis* cells stained .

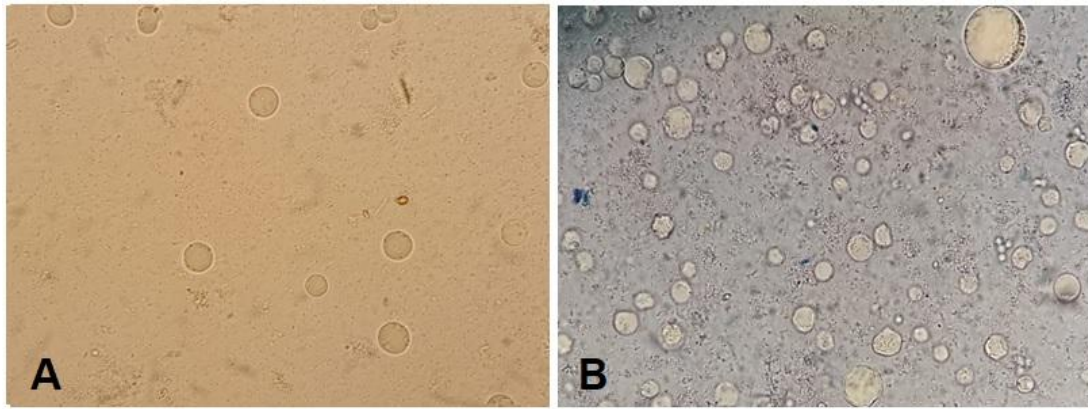


Figure 3. *Blastocystis* viability in the untreated control group, with trypan blue, at 40× magnification: (A) after 24 h incubation showing viable *Blastocystis* cells after 24 h, (B) after 96 h incubation showing continuous growth with morphologically intact cells.

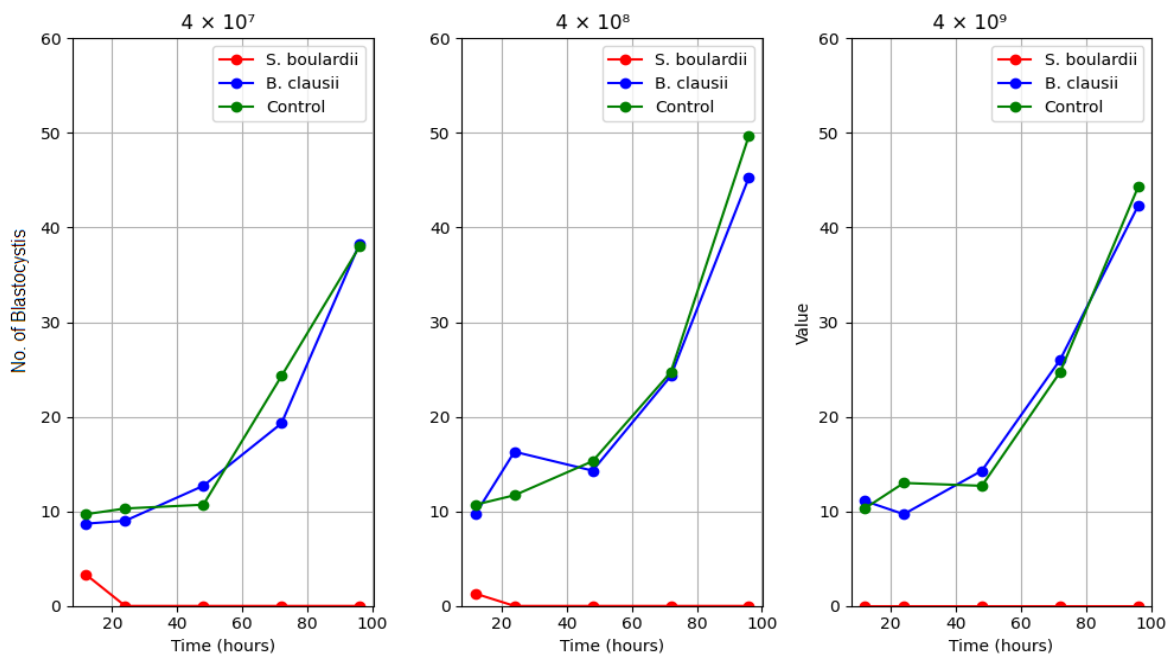


Figure 4. Time-dependent effects of *S. boulardii* and *B. clausii* on *Blastocystis* growth at three concentrations (4×10^7 , 4×10^8 , and 4×10^9 CFU/mL). Data were expressed as mean $\pm$ standard deviation (SD) of triplicate measurements. The y-axis represents the mean number of *Blastocystis*; the x-axis shows incubation time (hours).

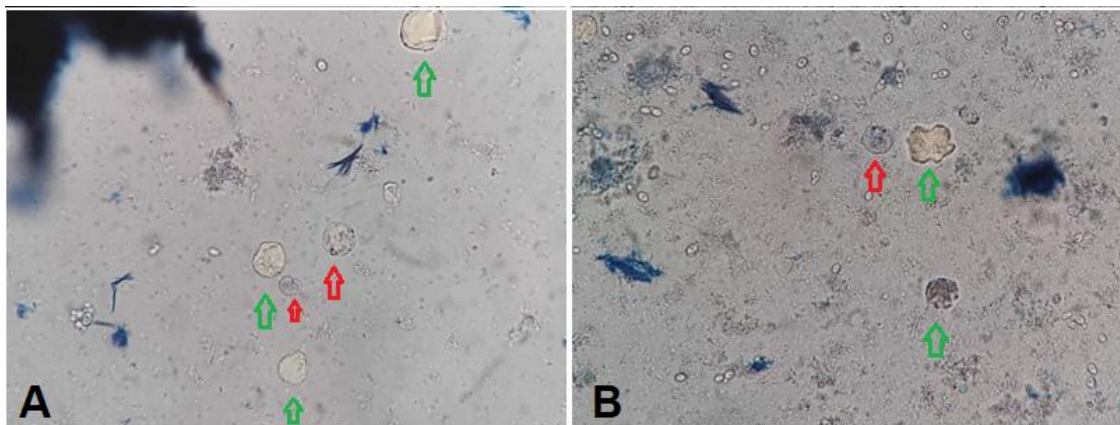


Figure 5. *Blastocystis* viability following treatment with 100 µg/mL metronidazole, trypan blue staining at 40× magnification: (A) after 12 h of treatment showing both viable and non-viable *Blastocystis* cells, non-viable cells (red arrows) and viable cells (green arrows), (B) after 96 h of treatment demonstrating trypan blue-stained non-viable cells (red arrows) and unstained but morphologically degenerated forms (green arrows).

Table 1. The overall *Blastocystis* numbers after co-incubation with *S. boulardii* and *B. clausii*

		12.hour	24.hour	48. hour	72.hour	96.hour
4x10 ⁷	<i>S. boulardii</i> ^a	3.3±0.6	0	0	0	0
	<i>B. clausii</i> ^b	8.7±2.9	9.0±2.6	12.7±2.5	19.3±1.2	38.3±2.1
	Control	9.7±0.6	10.3±2.5	10.7±2.9	24.3±3.2	38.0±2.6
4x10 ⁸	<i>S. boulardii</i> ^a	1.3±0.6	0	0	0	0
	<i>B. clausii</i> ^b	9.7±1.5	16.3±3.5	14.3±2.1	24.3±4.0	45.3±6.1
	Control	10.7±1.2	11.7±3.1	15.3±4.2	24.7±2.9	49.7±1.5
4x10 ⁹	<i>S. boulardii</i> ^a	0	0	0	0	0
	<i>B. clausii</i> ^b	11.1±1.0	9.7±2.1	14.3±2.1	26.0±5.6	42.3±2.1
	Control	10.3±1.5	13.0±2.6	12.7±1.2	24.7±4.2	44.3±3.2

Data were presented as mean ± standard deviation (SD). Statistical analyses were performed using the Kruskal–Wallis test followed by Dunn’s post-hoc test with Bonferroni correction. ^a significant difference compared with the control group at the same time point (P<0.05). ^b no significant difference compared with the control group (P>0.05).

4. Discussion

Although some recent studies suggest that *Blastocystis* may reduce beneficial gut bacteria, most research identifies it as a common component of a healthy gut microbiome (Deng and Tan, 2025). This discrepancy could stem from differences in study methodologies, population samples, and subtypes. In our study, *S. boulardii* inhibited *Blastocystis* growth, whereas *B. clausii* did not show such an effect. Supporting our findings on *S. boulardii*, a study on *Blastocystis*-infected children reported an efficacy of 99.4% compared to 77.3% for metronidazole (Dinleyici et al., 2011). A direct in vitro comparison between metronidazole and *S. boulardii* was not possible due to the challenge of detecting non-viable cells in the metronidazole-treated group. However, *S. boulardii* completely eradicated *Blastocystis* in the medium, while morphologically degenerated *Blastocystis* cells remained in the metronidazole group, supporting *S. boulardii* might be more effective than metronidazole. A case report further confirmed *S. boulardii*’s effectiveness and indicated that *Lactobacillus* and *Bifidobacterium* are ineffective in eradicating *Blastocystis* (Angelici et al., 2018). A study investigated therapeutic role of *S. boulardii* on *Blastocystis*. Treatment effectiveness was assessed through parasitological cure rate, histopathology and colonic mucosal expressions of proinflammatory cytokines (IL-6, IL-8, TNF-α) and iNOS. When combined with metronidazole it showed superior efficacy, achieving 100% reduction of *Blastocystis* in stool and improvement of tested parameters (Méabed et al., 2019). Although we found a complete inhibition of *Blastocystis* growth by *S. boulardii*, the underlying mechanisms remain to be clarified. Probiotic mechanisms of *S. boulardii* include luminal, mucosal, and trophic effects. Luminal activity inhibits pathogen toxins and inhibits growth by producing short chain fat acids (SCFAs). It also modulates gut microbial composition, raising symbiotic relationships and beneficial microbes for gut health (Ting et al., 2026). *Blastocystis* growth may be inhibited because of the reduction of luminal pH, competition for nutrients, and the production of metabolites (Plaza-Diaz et al., 2019).

A review of the literature revealed the lack of studies that investigate the effect of *Bacillus* species on *Blastocystis*, either in vitro or in vivo. They play crucial roles in enhancing beneficial flora, thereby contributing to human health including the competitive exclusion of pathogenic organisms, enhancement of nutritional value, improvement of dietary intolerances and allergies, anti-inflammatory and immune-modulatory effects, improved gut function, and reduced risk of cardiovascular disease and cancer (Payne, 2024). They can enhance the treatment of various gastrointestinal disorders, including acute gastroenteritis, antibiotic-associated diarrhea, Crohn’s disease, celiac disease, inflammatory bowel disease, *Clostridioides* and *Helicobacter pylori* infections, constipation, necrotizing enterocolitis, and colorectal cancer (Díaz-Madriz et al., 2023). A clinical study highlighted the effectiveness of *B. clausii* spores as a successful option for treating symptoms and as an immunomodulatory agent in the treatment of persistent diarrhea in children (Dang et al., 2024). Some other bacterial strains have been evaluated as probiotics in relation to *Blastocystis*. For example, *L. acidophilus* reduced intestinal inflammation and the side effects of metronidazole in *Blastocystis* infections (Abdeltawab et al., 2025). Both in vitro and in vivo studies have demonstrated that the combination of metronidazole and *Lactobacillus* probiotics effectively decreases the *Blastocystis* load, with the combination yielding the most significant parasitological and immunological improvements (Ma et al., 2025). Overall, the findings of this study support the hypothesis that an environment dominated by yeast inhibits the growth of *Blastocystis*.

A limitation of our study is that the probiotic microorganisms utilized were commercial products, which included additives. They introduce confounding variables, such as additives and inactive ingredients. This factor may have influenced the viability of *Blastocystis*. Additionally, our study focused on *Blastocystis* ST3, the most prevalent subtype in human fecal samples; however, other subtypes may exhibit different interactions with probiotics. A study showed that *Akkermansia muciniphila* was positively linked to all subtypes, while *Holdemania bififormis* was negatively

linked to them, and several bacteria were enriched in specific STs (Huang et al., 2024). Future research should consider using pure microorganisms and exploring subtype-dependent variations.

5. Conclusion

In conclusion, although the role of *Blastocystis* in gut health continues to be debated, our findings reveal that *S. boudardii* effectively inhibits its growth, while *B. clausii* shows no significant impact. Together with previous clinical and in vivo studies, our results suggest that *S. boudardii* could be a promising alternative or supportive option for managing *Blastocystis* infections. However, the mechanisms of *Blastocystis* growth inhibition after *S. boudardii* co-infection were not investigated in the present study. Possible mechanisms such as the secretion of inhibitory metabolites, competition for nutrients, or pH changes require further investigation. Future research should focus on these mechanisms, therapeutic potential, and clinical applications of *S. boudardii* in the treatment of *Blastocystis* infections.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	Z.E.D.	B.N.D.	A.S.D.	E.M.
C	20	20	20	40
D	30	20	20	30
S	15	15	15	55
DCP	25	25	25	25
DAI	25	25	25	25
L	30	20	20	20
W	20	20	20	40
CR	20	20	20	40
SR	20	20	20	40
PM	40	15	15	30
FA	40	15	15	30

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The research was conducted in accordance with the principles outlined in the Declaration of Helsinki. The procedures were approved by the Local Non-Invasive Ethics Committee of Aydin Adnan Menderes University, Faculty of Medicine (Approval date: April 29, 2025, protocol code: 2025/134).

Acknowledgements

This research was supported by TUBITAK 2209-A Research Project support program.

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References

- Abd Al-Khaliq, I. M., & Mohammed, S. T. (2015). Efficiency of *Saccharomyces boudardii* on *Blastocystis hominis* in laboratory mice. *World Journal of Pharmacological Research*, 4(6), 64-73.
- Abdeltawab, M. S. A., Abdel Aal, S. M., Ali, S. M., Zaki, K. Y. W., Saad El-Din, S., & Amin, N. M. (2025). Anti-inflammatory effect of probiotics in patients with *Blastocystis* spp. infection. *Cytokine*, 195, 157013. <https://doi.org/10.1016/j.cyto.2025.157013>
- Alsalemi, W., Chik, Z., & Alshawsh, M. A. (2025). Probiotic bacteria vs. yeast for gastrointestinal diseases treatment: Protocol for a systematic review and meta-analysis. *Plos One*, 20(6), e0324926. <https://doi.org/10.1371/journal.pone.0324926>
- Angelici, M. C., Nardis, C., Scarpelli, R., & Ade, P. (2018). *Blastocystis hominis* transmission by non-potable water: A case report in Italy. *The New Microbiologica*, 41(2), 173-177.
- Aykur, M., Karakavuk, E., Karakavuk, M., Akil, M., Can, H., Döşkaya, M., & Dağcı, H. (2020). Inhibitory effect of Tunceli garlic (*Allium tuncelianum*) on *Blastocystis* subtype 3 grown in vitro. *Expert Opinion on Orphan Drugs*, 8(11), 489-496. <https://doi.org/10.1080/21678707.2020.1857725>
- Aykur, M., Malatyali, E., Demirel, F., Cömert-Koçak, B., Gentekaki, E., Tsaousis, A. D., & Dogruman-Al, F. (2024). *Blastocystis*: A mysterious member of the gut microbiome. *Microorganisms*, 12(3), 461. <https://doi.org/10.3390/microorganisms12030461>
- Dang, H. T., Tran, D. M., Phung, T. T. B., Bui, A. T. P., Vu, Y. H., Luong, M. T., Nguyen, H. M., Trinh, H. T., Nguyen, T. T., Nguyen, A. H., & Van Nguyen, A. T. (2024). Promising clinical and immunological efficacy of *Bacillus clausii* spore probiotics for supportive treatment of persistent diarrhea in children. *Scientific Reports*, 14(1), 6422. <https://doi.org/10.1038/s41598-024-56627-9>
- Deng, L., & Tan, K. S. W. (2025). From parasite to partner: Unravelling the multifaceted role of *Blastocystis* in human health and disease. *The Lancet Microbe*, 6(9), 101155. <https://doi.org/10.1016/j.lanmic.2025.101155>
- Díaz-Madriz, J. P., Zavaleta-Monestel, E., Rojas-Chinchilla, C., Arguedas-Chacón, S., Serrano-Arias, B., Ferreto-Meza, M. A., Romero-Chavarría, B. M., Zumbado-Amerling, P., Vásquez-Mendoza, A. F., Gutiérrez-González, K. S., & Rodríguez, C. (2023). Bacteremia following *Alkalihalobacillus clausii* (formerly *Bacillus clausii*) administration in immunosuppressed adults: A case series. *Bacteria*, 2(4), 185-195. <https://doi.org/10.3390/bacteria2040014>
- Dinleyici, E. C., Eren, M., Dogan, N., Reyhanioglu, S., Yargic, Z. A., & Vandenplas, Y. (2011). Clinical efficacy of *Saccharomyces boudardii* or metronidazole in symptomatic children with *Blastocystis hominis* infection. *Parasitology Research*, 108(3), 541-545. <https://doi.org/10.1007/s00436-010-2095-4>
- Genç, S., & Soysal, M. İ. (2018). Parametric and nonparametric post hoc tests. *Black Sea Journal of Engineering and Science*, 1(1), 18-27. <https://izlik.org/JA73XC42GW>
- Ghelardi, E., Abreu Y Abreu, A. T., Marzet, C. B., Álvarez Calatayud, G., Perez, M., & Moschione Castro, A. P. (2022). Current progress and future perspectives on the use of *Bacillus clausii*. *Microorganisms*, 10(6), 1246. <https://doi.org/10.3390/microorganisms10061246>
- Huang, L. S., Yeh, Y. M., Chiu, S. F., Huang, P. J., Chu, L. J., Huang, C. Y., Cheng, F. W., Chen, L. C., Lin, H. C., Shih, Y. W., Lin, W. N., & Huang, K. Y. (2024). Intestinal microbiota analysis of different *Blastocystis* subtypes and *Blastocystis*-negative individuals in Taiwan. *Biomedical Journal*, 47(4), 100661.

- <https://doi.org/10.1016/j.bj.2023.100661>
- Lepczyńska, M., & Dzika, E. (2019). The influence of probiotic bacteria and human gut microorganisms causing opportunistic infections on Blastocystis ST3. *Gut Pathogens*, 11, 6. <https://doi.org/10.1186/s13099-019-0287-8>
- Ma, P., Mu, W., Wang, Y., Liu, Y., Zou, Y., Lu, Z., Pang, S., Pan, H., Zhang, L., Chen, L., Yang, Y., Lin, X., Kuang, Z., Luo, W., Liu, G., & Wang, S. (2025). Blastocystis load mediates the gut microbiome associations with within-host diversity of Blastocystis in non-human primates. *ISME Communications*, 5(1), ycaf170. <https://doi.org/10.1093/ismeco/ycaf170>
- Mandal, S., Mondal, C., & Lyndem, L. M. (2024). Probiotics: An alternative anti-parasite therapy. *Journal of Parasitic Diseases*, 48(3), 409–423. <https://doi.org/10.1007/s12639-024-01680-4>
- Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., & Rotondo, J. C. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*, 12(1), 184. <https://doi.org/10.3390/cells12010184>
- Méabed, E. M. H., Abdelhafez, D. N., & Abdelaliem, Y. F. (2019). Saccharomyces boulardii inhibits the expression of pro-inflammatory cytokines and inducible nitric oxide synthase genes in the colonic mucosa of rats experimentally infected with Blastocystis subtype-3 cysts. *Parasitology*, 146(12), 1532–1540. <https://doi.org/10.1017/S0031182019000696>
- Önder, H. (2018). Nonparametric statistical methods used in biological experiments. *Black Sea Journal of Engineering and Science*, 1(1), 1-6. <https://izlik.org/JA84XN76YH>
- Pawelec-Peciak, O., Lanocha-Arendarczyk, N., Grzeszczak, K., & Kosik-Bogacka, D. (2025). The role of Blastocystis spp. in the etiology of gastrointestinal and autoimmune diseases. *Pathogens*, 14(4), 313. <https://doi.org/10.3390/pathogens14040313>
- Payne, J., Bellmer, D., Jadeja, R., & Muriana, P. (2024). The potential of Bacillus species as probiotics in the food industry: A review. *Foods*, 13(15), 2444. <https://doi.org/10.3390/foods13152444>
- Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances in Nutrition*, 10(1), S49–S66. <https://doi.org/10.1093/advances/nmy063>
- Ribeiro, M. R. S., Oliveira, D. R., Oliveira, F. M. S., Caliari, M. V., Martins, F. S., Nicoli, J. R., Torres, M. F., Andrade, M. E. R., Cardoso, V. N., & Gomes, M. A. (2018). Effect of probiotic Saccharomyces boulardii in experimental giardiasis. *Beneficial Microbes*, 9(5), 789–797. <https://doi.org/10.3920/BM2017.0155>
- Roberts, T., Bush, S., Ellis, J., Harkness, J., & Stark, D. (2015). In vitro antimicrobial susceptibility patterns of Blastocystis. *Antimicrobial Agents and Chemotherapy*, 59(8), 4417–4423. <https://doi.org/10.1128/AAC.04832-14>
- Scicluna, S. M., Tawari, B., & Clark, C. G. (2006). DNA barcoding of Blastocystis. *Protist*, 157(1), 77–85. <https://doi.org/10.1016/j.protis.2005.12.001>
- Sejnohova, A., Koutenska, M., Jirku, M., Brozova, K., Pavlickova, Z., Kadlecova, O., Cinek, O., Maloney, J. G., Santin, M., Petrzelkova, K. J., & Jirku, K. (2024). A cross-sectional survey of Blastocystis sp. and Dientamoeba fragilis in non-human primates and their caregivers in Czech zoos. *One Health*, 19, 100862. <https://doi.org/10.1016/j.onehlt.2024.100862>
- Tan, K. S. (2008). New insights on classification, identification, and clinical relevance of Blastocystis spp. *Clinical Microbiology Reviews*, 21(4), 639–665. <https://doi.org/10.1128/CMR.00022-08>
- Ting, T. Y., Lee, W. J., & Goh, H. H. (2026). Molecular genetics and probiotic mechanisms of Saccharomyces cerevisiae var. boulardii. *Probiotics and Antimicrobial Proteins*, 18(2), 3040–3054. <https://doi.org/10.1007/s12602-025-10634-y>
- Tomičić, Z., Šarić, L., & Tomićić, R. (2024). Novel insights in the application of probiotic yeast Saccharomyces boulardii in dairy products and health promotion. *Foods*, 13, 2866. <https://doi.org/10.3390/foods13182866>
- Uluslan Bağcı, Ö., & Aral Akarsu, G. (2025). A systematic review about the efficacy of antiparasitic agents in the treatment of Blastocystis species. *Acta Parasitologica*, 70(6), 208. <https://doi.org/10.1007/s11686-025-01145-5>
- Yoshikawa, H., & Iwamasa, A. (2016). Human Blastocystis subtyping with subtype-specific primers developed from unique sequences of the SSU rRNA gene. *Parasitology International*, 65(6), 785–791. <https://doi.org/10.1016/j.parint.2016.03.002>