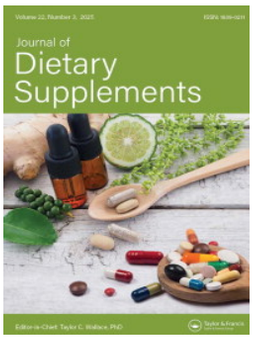

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RESEARCH ARTICLE



# A Mushroom Based Prebiotic Supplement Pilot Study Among Patients with Crohn's Disease

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**ABSTRACT**

Data on a mushroom based prebiotic supplementation in patients with Crohn's disease (CD) in western population is scarce. In this pilot trial, we aimed to assess the clinical efficacy and fecal microbial compositional and functional alterations associated with 'Mycodigest,' a commercial prebiotic supplement composed of three mushroom extracts. Patients with mild to moderate CD were recruited to a single center, randomized, double-blind, placebo-controlled pilot induction trial. Clinical efficacy using the Harvey-Bradshaw index and biochemical response using C-reactive protein and fecal calprotectin were assessed at week 8 post-intervention. Fecal samples were assessed by DNA shotgun metagenomic sequencing. A multivariable linear mixed effects model was used to assess alteration in fecal microbiome composition and function pre- and post-'Mycodigest' intervention. Clinical response was higher in the 'Mycodigest' intervention ($N=10$) compared to the placebo ($N=6$) group (80 *vs.* 16.7%, respectively, $p=0.035$). There were no differences in terms of biochemical response within each group pre- and post-intervention. Post-'Mycodigest' intervention, 25 species were found to be differentially abundant compared to baseline, including increase in short chain fatty acid producing bacteria, such as *Parabacteroides distasonis* (Beta coefficient 0.92, 95% Confidence interval [CI] 0.36–1.47) and *Faecalimonas umbilicata* (Beta coefficient 0.57, 95% CI 0.23–0.90). Two microbial pathways related to the metabolism of isoprenoid compounds were increased post-'Mycodigest' intervention. Mushroom based prebiotic supplementation in subjects with CD resulted in clinical improvement which may be related to post-intervention favorable compositional and functional microbial alterations.

**KEYWORDS**

C-reactive protein; Crohn's disease; fecal calprotectin; gastrointestinal microbiome; mushroom; prebiotic

## Introduction

Despite a wide variety of advanced medical treatments for Crohn's disease (CD), their overall efficacy is limited, and there is an emerging need for additional therapeutic strategies (Kumar et al. 2022; Chang et al. 2024). The gut microbiome, including

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bacterial, mycobial, and viral communities, has a crucial role in the pathogenesis of CD (Pascal et al. 2017; Raygoza Garay et al. 2023), and thus therapeutic approaches that target the gut microbiome, such as dietary interventions, fecal microbial transplantation, probiotics, and prebiotics are being investigated (Yanai et al. 2022; Gowen et al. 2023).

Prebiotics are dietary supplements, typically fibers, that nourish the human gut microbiota to promote host health (Gibson et al. 2017). Mouse studies have shown that prebiotics administration increased the abundance of beneficial bacteria, including *Bifidobacterium* and *Lactobacillus* (Osman et al. 2006), known to be depleted in CD, and led to reduction in plasma pro-inflammatory cytokines, such as IL-6 and to increased expression of intestinal tight junction proteins (Wong et al. 2022).

In humans, several studies have investigated the effects of administration of the prebiotic oligofructose on gut health. In two studies by De Preter et al. healthy subjects who received oligofructose-enriched inulin prebiotic showed a decrease in the formation of toxic metabolites in the colon and increase in total fecal bifidobacteria (De Preter et al. 2007, 2008). Specifically in CD, a study by Anderson et al. showed that decreased intake of the prebiotics fructans and oligofructose, was correlated with more severe abdominal pain and poorer well-being (Anderson et al. 2015).

Mushrooms can be considered a potential source of prebiotics as they contain different polysaccharides, such as mannans, chitin, and galactans (Singdevsachan et al. 2016). 'Mycodigest' is a prebiotic supplement which includes three mushroom extracts, i.e. *Trametes versicolor* (commonly known as turkey tail mushroom), *Hericium erinaceus* (commonly known as lion's mane mushroom), and *Agaricus blazei* Murill (commonly known as almond mushroom). Previous studies have demonstrated beneficial gut health properties of mushroom based prebiotics, potentially *via* effect on oxidative stress reduction, promotion of gut barrier integrity, inhibition of pro-inflammatory cytokines, and influence on gut microbiota composition (Ren et al. 2018; Wang et al. 2021; Impellizzeri et al. 2022; Ji et al. 2023). In two placebo controlled studies by Therkelsen et al. intake of *A. blazei* Murill-based mushroom extract, resulted in beneficial symptomatic effect in patients with CD and ulcerative colitis compared to placebo (Therkelsen et al. 2016a, 2016b). However, it remains to be determined whether this potential clinical benefit in CD is also associated with related changes to the gut microbiome.

In this single center, randomized, double-blind, placebo-controlled pilot trial we aimed to evaluate the impact of 'Mycodigest' supplementation on clinical and biochemical parameters as well as on the fecal microbial composition and function.

## Methods

### *Patient population*

Adult patients with CD were recruited in the Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, to a randomized, double-blind, placebo-controlled pilot trial.

Patients could be included if they were 18–70 years of age, had an established diagnosis of CD, had mild to moderate active disease defined as Harvey-Bradshaw index (HBI) score >4 and <16, or HBI <4 and calprotectin >250, on stable medical treatment before enrollment (mesalamine at least 6 weeks, or steroids at least 2 weeks, or

immunomodulator at least 12 weeks or biologics at least 12 weeks) and throughout the study period and signed informed consent. Patients were excluded if they had current gut infection, such as *Clostridioides difficile* infection, positive stool culture or parasites, antibiotic use during participation in the study, had chronic conditions, such as cancer, organ transplantation, advanced kidney or liver disease, systemic inflammatory conditions other than inflammatory bowel disease (IBD), were pregnant, had an ileostomy, pouch or short bowel.

After screening, 19 subjects were recruited to the study and underwent randomization. Three subjects dropped from the study following randomization (one subject started taking antibiotics for an ear infection, the second subject was diagnosed with breast cancer right after recruitment and the third subject decided to withdraw for a non-medical issue) leaving 10 subjects in the 'Mycodigest' supplementation group and six in the placebo group (Supplementary Figure 1).

### Intervention

'Mycodigest' supplementation and placebo were manufactured by 'Mycolivia Medicinal Mushrooms' LTD, to be identical in size, shape, and color. 'Mycodigest' is a dietary supplement which consists of traditional medicinal mushrooms, as essences and grounded powder. These include *T. coriolus versicolor*, *H. erinaceus*, and *A. blazei* Murill (Supplementary Appendix A). A 10% powder concentrate of Reishi (Ganoderma Lucidum) mushroom was added to the placebo pills to achieve similar smell to that of the 'Mycodigest' supplement. Compliance to treatment was considered as taking 80% of supplement/placebo treatment and was monitored by telephone calls and emails to patients during the study phase, and by counting the pills which were not taken at the end of the trial.

Treatment with 'Mycodigest' supplement/placebo was titrated with patients receiving an initiation dose of 2 pills/day for 7 days, and then gradually increased to 4 pills/day for 7 days and then 6 pills/day for 42 days. Thus, the full dose of the treatment was administered for 6 wk. Patients were instructed to ingest the capsules in divided dose of 2 capsules with or right after each meal.

During the study period the medical care-givers, who were blinded to treatment allocation, were able to provide the same standard IBD medical care, nutritional counseling, and other supporting therapies.

The study [ClinicalTrials.gov registration: NCT04329481] was approved by the local ethics committee in the Tel Aviv Sourasky Medical Center [IRB #0643-17-TLV]. Due to the covid-19 pandemic during the study period, restricting patients visits and sample collections, the study was prematurely terminated and reframed as a pilot study.

### Clinical and biochemical outcomes

Clinical outcomes were measured at week 8 post- intervention by the HBI score, which is composed several variables representing the previous day, as reported by the patient, and include the following: (a) general well-being; (b) abdominal pain; (c) number of liquid/soft stools; (d) abdominal mass on physical examination and (e) complications which include extraintestinal manifestation, anal fissure, new fistula or abscess.

Clinical response was defined as a decrease in HBI score of ≥ 3 points from baseline, whereas clinical remission was defined as HBI score ≤4 (Marín-Jiménez et al. 2022). Biochemical activity was separately assessed pre-and post-intervention within each group by serum C-reactive protein and by fecal calprotectin. Fecal calprotectin data were available for 9 out of 10 participants in the 'Mycodigest' group and for 5 out of 6 participants in the placebo group.

### Stool sampling, DNA extraction, and metagenomic sequencing

Stool samples were self-collected in sterile cups either at our center or at home in the morning before the day of the visit. In case the sample was collected at home, the cup was stored on ice and brought to our center within 4 h. All samples were immediately stored in −80 °C degrees until processed and analyzed. All the samples (baseline and week 8) were processed together at the end of the study to avoid potential technical batch effect.

Fecal microbial DNA was purified using DNeasy PowerMag Soil DNA extraction kit [Qiagen] optimized for Tecan automated platform. Next-generation sequencing [NGS] libraries were prepared using Nextera DNA library prep [Illumina] and sequenced on a NovaSeq sequencing platform [Illumina]. Sequencing was performed with 75-bp single-end reads with the depth of 10 million reads per sample. Metagenomic reads containing Illumina adapters, filtered low-quality reads, and trimmed low-quality read edges, were filtered. Host DNA was detected by mapping with Bowtie to the human genome with inclusive parameters and removed those reads. Bacterial relative abundance estimation was performed by mapping bacterial reads to species-level genome bins [SGB] representative genomes (Pasolli et al. 2019). All SGB representatives with at least 5 genomes in a group were selected, and for these representatives' genomes, only unique regions as a reference dataset were kept. Mapping was performed using Bowtie (Langmead and Salzberg 2012), and abundance was estimated by calculating the mean coverage of unique genomic regions across the 50% most densely covered areas, as previously described (Korem et al. 2015).

HUMAnN3.6 (v0.11.2) software was used to identify the taxonomic and functional profiles of each community, using MetaPhlAn3.0 (v2.7.8) for taxonomy and the UniRef90 database for function, following all default parameters (Abubucker et al. 2012; Suzek et al. 2015). Resulting functional annotations were mapped to the MetaCyc gene family ontology (Caspi et al. 2016).

### Assessment of fecal calprotectin

Fecal calprotectin concentrations were analyzed by the Liaison XL machine using the designated CLIA Liaison-calprotectin kit (Diasorin, Saluggia, Italy) according to manufacturer instructions.

### Statistical analysis

For the clinical data, the Shapiro-Wilk test was used to determine normality of outcomes-related data. All continuous and ordinal outcomes data (fecal calprotectin,

CRP, HBI, stool frequency, and abdominal pain scores) were non-normally distributed, and as such the Wilcoxon signed-rank test was used for these comparisons within the groups. The Fisher's Exact test was used to compare categorical data (clinical response and remission) between groups. $p$-Value <0.05 was considered significant.

For the microbiome data, to assess alpha diversity, species level Shannon index was compared between pre- and post-'Mycodigest' intervention using the Wilcoxon rank sum test. Beta diversity was assessed with principal coordinates analysis plots at the species level based on Bray-Curtis dissimilarity index to assess post-'Mycodigest' intervention microbial community shifts. Permutation analysis of variance (PERMANOVA) was applied using the adonis function on distance matrices with 1000 permutations. Subjects ID's were included as strata to account for the paired pre- and post-intervention samples from the same subjects (vegan (v2.5-7) R package).

A multivariable linear mixed effects model (multivariate analysis by linear models [MaAsLin2] (v1.4.0) R package (Mallick et al. 2021)) was used to assess associations between 'Mycodigest' intervention with changes in individual gut microbial species and metabolic pathways. The effect size (beta-coefficients) generated by the model, represents the average change in the relative abundance of the feature between the pre- and post-intervention state. The model filtering parameters were set to include only features with minimum prevalence of 20% and minimum abundance higher than 0.01%. Age, sex, and body-mass-index were included as co-variates in all models. To account for pre-and post-intervention samples from the same subject, subjects' IDs were included in the model as random effects.

For the microbial compositional data, after the removal of singletons, data was converted to relative abundance, and agglomeration to species level was performed. After applying filtering steps, 95 species level taxa were included in the analysis. For the microbial functional analysis, the relative abundances of the unstratified MetaCyc metabolic pathways were analyzed. After applying filtering steps, 185 pathways were included in the analysis.

MaAsLin2 accounts for multiple testing using Benjamini-Hochberg correction. Significant associations were considered for features with $p$-values <0.05 and false discovery rates (FDR) values <0.2 (Benjamini and Hochberg 1995; Mallick et al. 2021).

## Results

### *Cohort description*

The cohort of patients with Crohn's disease included 10 subjects in the 'Mycodigest' group and six subjects in the placebo group. The median age of the entire cohort was 32.5 years (Interquartile range [IQR] [29.7–42.5]), and 50% were females. There were no significant differences between the groups in age, gender, body mass index, smoking status, disease duration, disease location and behavior, baseline clinical activity, and inflammatory markers of C-reactive protein and fecal calprotectin (Table 1). To note, three patients in the 'Mycodigest' group were on biologic treatment with adalimumab compared to none in the Placebo group, however, all three were on stable treatment at the time of recruitment and continued the treatment throughout the study (Table 1).

**Table 1.** Cohort demographics and clinical data.

| | Mycodigest ($N=10$) | Placebo ($N=6$) | $p$-Value |
|---|---|---|---|
| Gender, females (%) | 6 (60.0%) | 2 (33.3%) | 0.61 |
| Age, median years [IQR] | 32.5 [26.0; 42.0] | 33.5 [31.0; 65.0] | 0.62 |
| BMI, median kg/m$^2$ [IQR] | 23.6 [20.5; 25.8] | 22.3 [20.7; 25.6] | 0.90 |
| Disease duration, median years (IQR) | 9.0 [4.0; 16.0] | 14.0 [1.0; 25.0] | 0.99 |
| Smoking, *n* (%) | | | 0.051 |
| Non-smoker | 7 (70.0%) | 2 (33.3%) | |
| Active smoker | 2 (20.0%) | 0 (0%) | |
| Past smoker | 1 (10.0%) | 4 (66.7%) | |
| CD Montreal location, *n* (%) | | | 0.11 |
| Ileum (L1) | 3 (30.0%) | 4 (66.7%) | |
| Colon (L2) | 2 (20.0%) | 2 (33.3%) | |
| Ileo-colonic (L3) | 5 (50.0%) | 0 (0%) | |
| CD Montreal behavior, *n* (%) | | | 0.41 |
| Inflammatory (B1) | 8 (80.0%) | 4 (66.7%) | |
| Stricturing (B2) | 1 (10.0%) | 2 (33.3%) | |
| Penetrating (B3) | 1 (10.0%) | 0 (0%) | |
| CD perianal disease, *n* (%) | 3 (30.0%) | 1 (20.0%) | 0.99 |
| Current treatment, *n* (%) | | | |
| No treatment | 4 (40.0%) | 2 (33.3%) | 0.99 |
| Mesalamine | 2 (20.0%) | 1 (16.7%) | 0.99 |
| Infliximab | 0 (0%) | 0 (0%) | 1.00 |
| Adalimumab | 3 (30.0%) | 0 (0%) | 0.41 |
| Vedolizumab | 0 (0%) | 0 (0%) | 1.00 |
| Steroids | 0 (0%) | 0 (0%) | 1.00 |
| Biologic naive, *n* (%) | 6 (60.0%) | 4 (66.7%) | 0.99 |
| Harvey Bradshaw Index, baseline, median [IQR] | 7.0 [5.0; 10.0] | 5.0 [3.0; 6.0] | 0.053 |
| Fecal calprotectin, baseline (µg/g), median [IQR] | 135.5 [93.0; 411.0] | 316.5 [190.0; 461.0] | 0.37 |
| C-reactive protein, baseline (mg/dL), median [IQR] | 3.0 [0.8; 4.8] | 6.3 [5.0; 9.8] | 0.12 |

IQR: interquartile range; BMI: body mass index; CD: Crohn's disease.

Demographic and clinical data for the prebiotic and placebo intervention groups. No statistically significant differences were observed for any of the variables between the groups. Comparison of categorical variables was performed using the $\chi^2$ test. For continuous variables, Kruskal-Wallis or Wilcoxon signed-rank tests were applied to compare the medians.

## *Subjects in the 'Mycodigest' group achieved higher rates of clinical response compared to placebo*

Clinical response at 8 weeks occurred in 80% of the patients in the 'Mycodigest' group *versus* 16.7% in the placebo group ($p=0.035$). Clinical remission rates at week 8 were also higher in the 'Mycodigest' compared to the placebo group, 60 and 16.7%, respectively, however, this difference was not statistically significant ($p=0.14$) (Figure 1). For the specific components of the HBI, in terms of the outcome of abdominal pain, at week 8, in the Mycodigest group, 3 patients (30%) reported an improvement, 7 patients (70%) reported no change and none reported worsening of their abdominal pain. Contrarily, in the placebo group, only one patient (16.7%) reported an improvement, 2 patients (33.3%) reported no change and 3 patients (50%) reported worsening of their abdominal pain. However, these observed changes between the groups did not reach statistical significance (Figure 2(A)). In terms of the number of liquid/soft stools per day, a significant decrease from median 3.5 [IQR 2.0–5.0] stools/day, at week 0 to 1.0 [IQR 0–4.75] stools/day, at week 8 ($p=0.0284$) in the Mycodigest group was observed, while no significant change was observed in the placebo group with 3.0 [IQR 1.25–4.0] stools/day at week 0, compared to 2.5 [IQR 1.0–4.0] stools/day at week 8 ($p=0.99$) (Figure 2(B)).

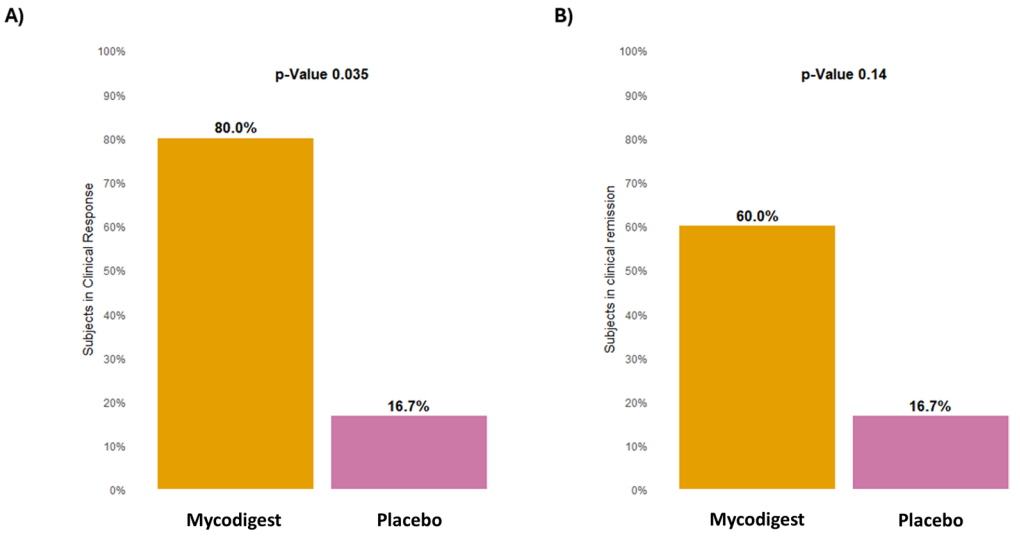


**Figure 1.** Clinical response and remission outcome between the 'Mycodigest' and placebo intervention groups. Stacked bar plots comparing the proportion of clinical response (A) and clinical remission (B) between the 'Mycodigest' prebiotic (yellow color) and placebo (purple color) intervention groups. Fisher's exact test was used to compare both groups with $p$-value < 0.05 considered significant.

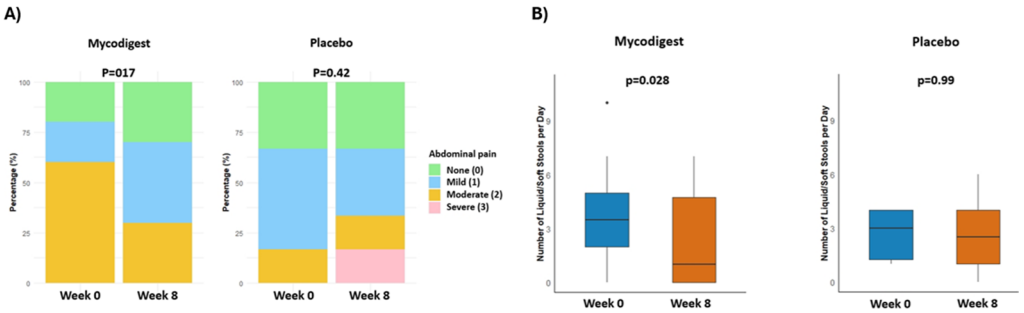


**Figure 2.** Pre- and post-intervention changes in abdominal pain and number of liquid stools per day scores in the 'Mycodigest' and placebo groups. Changes in the abdominal pain scores (A) and in the number of liquid stools per day (B) in the 'Mycodigest' and placebo groups pre- and post-intervention, at week 0 and week 8, respectively. The Wilcoxon signed-rank test was used with $p$-value < 0.05 considered significant.

No statistically significant changes in either C-reactive protein or fecal calprotectin levels were noted in either group comparing week 0 to week 8 levels (Supplementary Figures 2 and 3, respectively).

## *'Mycodigest' intervention was associated with individual microbial species alterations*

We next performed a paired metagenomic analysis comparing the microbial compositional changes pre- and post-'Mycodigest' intervention. No differences in either alpha diversity (Shannon index, $p = 0.68$) or in beta diversity (Bray Curtis index, $p = 0.19$)

were noted at week 8 following 'Mycodigest' supplementation (Figures 3(A,B), respectively). We identified 25 individual species that were differentially abundant at week 8 post-intervention ($p < 0.05$ and $q < 0.2$) (Supplementary Table 1). Among those species, *Clostridium* sp. *AF34 10BH* (Beta coefficient 1.75, 95% Confidence interval [CI] 0.93–2.57), *Parabacteroides distasonis* (Beta coefficient 0.92, 95% CI 0.36–1.47), *Faecalimonas umbilicata* (Beta coefficient 0.57, 95% CI 0.23–0.90), and *Sutterella wadsworthensis* (Beta coefficient 0.56, 95% CI 0.25–0.87) were significantly increased, whereas *Lachnospira* sp. *NSJ 43* (Beta coefficient −1.45, 95% CI −2.34 to −0.55), *Clostridiales bacterium KLE1615* (Beta coefficient −1.41, 95% CI −2.15 to −0.67), *Blautia* sp. *AF19 10LB* (Beta coefficient −1.03, 95% CI −1.38 to −0.68), and *Clostridiaceae bacterium* (Beta coefficient −0.57, 95% CI −0.95 to −0.19) were significantly decreased post-intervention at week 8 ($q < 0.05$ for all) (Figure 3(C)).

In an exploratory analysis we observed a non-significant numerical increase in alpha diversity (Shannon index, $p = 0.57$) and only a trend toward significance in microbial compositional shifts (Bray Curtis index, PERMANOVA: $R^2 = 0.19$, $p = 0.08$) when comparing subjects who achieved clinical remission at week 8 ($N = 5$) *versus* those who did not ($N = 3$) in the 'Mycodigest' group intervention (Supplementary Figure 4).

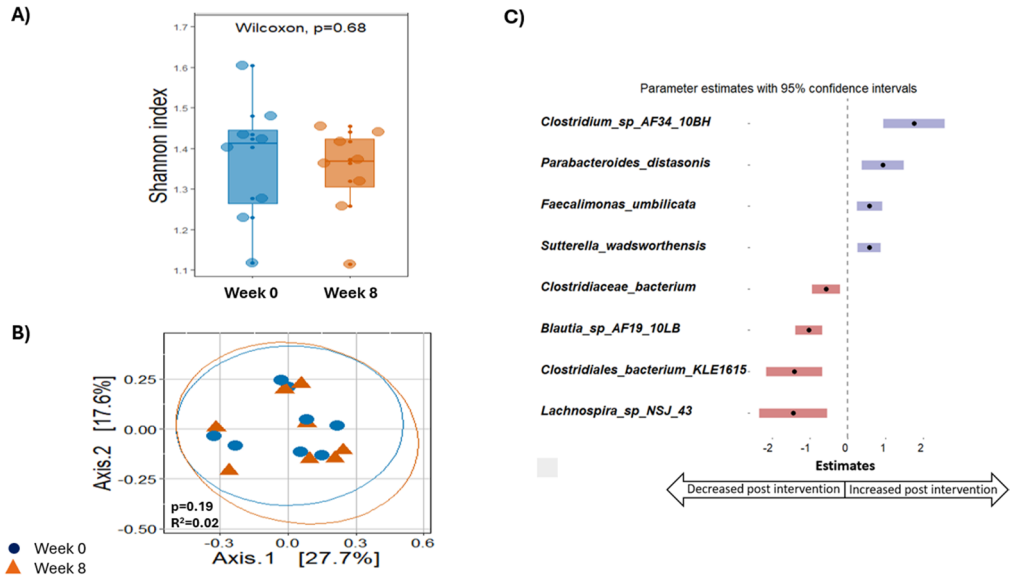


**Figure 3.** Diversity and species differential abundance in the 'Mycodigest' intervention group pre- and post-intervention. (A) Alpha diversity pre- and post-'Mycodigest' supplementation (blue and orange colors, respectively) expressed by the Shannon index. *p*-Value is calculated using the Wilcoxon test. (B) Beta diversity assessed with principal coordinates analysis plots at the species level based on Bray-Curtis dissimilarity index. Ellipsoids represent a 95% confidence interval (blue = pre-'Mycodigest' supplementation; orange = post-'Mycodigest' supplementation). Permutation analysis of variance (PERMANOVA) was applied on distance matrices with 1000 permutations. (C) Coefficient plot showing the effect size and 95% confidence interval (x-axis) for the species found to be differentially abundant post-'Mycodigest' intervention (y-axis), based on MaAsLin2 adjusted for age, gender and body mass index. Only species with *q*-values < 0.05 are shown (for the full list of species with *p*-value < 0.05 and *q*-values < 0.2 see Supplementary Table 1). Purple color denotes taxa increased post-'Mycodigest' intervention and pink color denotes taxa decreased post-'Mycodigest' intervention.

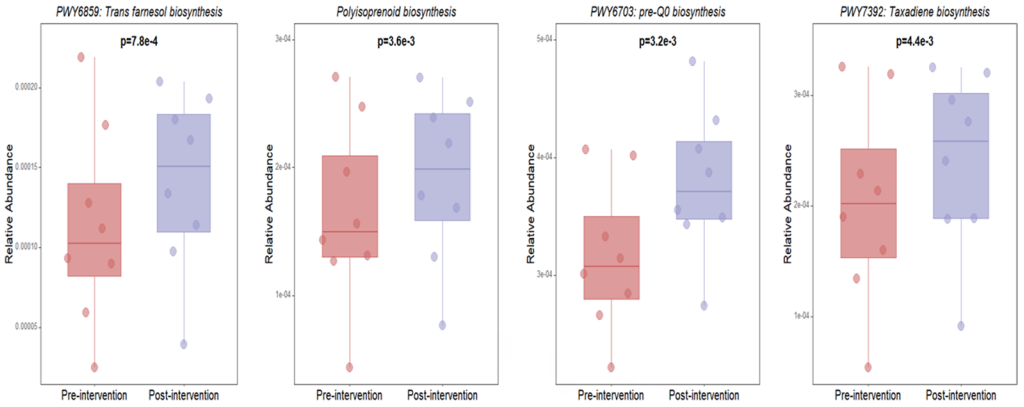


**Figure 4.** Microbial MetaCyc pathways differential abundance in the 'Mycodigest' intervention group pre- and post-intervention. Box plots showing the comparison between the relative abundances of microbial MetaCyc pathways associated with pre-and post-'Mycodigest' prebiotic intervention. *q*-values generated by MaAsLin2 regression models (see Methods).

### *'Mycodigest' supplementation led to alterations in microbial metabolic pathways related to farnesol and polyisoprenoid biosynthesis*

We next assessed the effect of 'Mycodigest' supplementation on the microbial functional capacity. We identified four metabolic pathways that were all significantly increased post-intervention (Supplementary Table 2). Two pathways were related to the metabolism of isoprenoid compounds, Including PWY6859: all-trans-farnesol biosynthesis (Beta coefficient 0.29, 95% CI 0.12–0.52) and polyisoprenoid biosynthesis (*Escherichia coli*) (Beta coefficient 0.15, 95% CI 0.05–0.25) (Figure 4).

## Discussion

In this pilot study, we found that patients with clinically active CD who received 'Mycodigest' prebiotic supplementation containing three mushroom extracts of *T. coriolus versicolor*, *H. erinaceus*, and *A. blazei* Murill, showed higher rates of clinical response compared to placebo. Moreover, the prebiotic intervention was associated with microbial compositional alterations toward more beneficial taxa and with microbial functional shifts toward mycobial related pathways.

Data on the use of mushrooms based prebiotics in CD is scarce, and a limited number of human studies from western countries have examined its potential beneficial effect (Førland et al. 2011; Therkelsen et al. 2016a). Therkelsen et al. showed that patients with CD who received AndoSan™, an *A. blazei* Murill-based mushroom extract, improved symptomatically compared to baseline. However, when compared to the placebo group, no differences were noted in terms of symptoms, fatigue, quality of life, and fecal calprotectin (Therkelsen et al. 2016a). In our study, we showed that the three mushrooms extract based 'Mycodigest' prebiotic intervention was associated with symptomatic improvement compared to placebo, but similarly to the study by Therkelsen et al., this was not associated with more stringent end points of C-reactive protein or fecal calprotectin improvement within each group comparing pre-and post-intervention.

This may suggest that this symptomatic improvement is mediated *via* other biological pathways not directly related to decrease in inflammatory burden. Alternatively, this may be the result of the small cohort or could signify a longer duration required for biomarker responses.

We next assessed the 'Mycodigest' prebiotic intervention effect on fecal microbial composition. Previous studies demonstrated the potential prebiotic effects of *T. coriolus versicolor* (Yu et al. 2013; Pallav et al. 2014), *H. erinaceus* (Diling et al. 2017; Li et al. 2021), and *A. blazei* Murill (Zhao et al. 2024) by promoting the growth of beneficial taxa. These beneficial microbial effects were mainly exerted *via* polysaccharides, the major active ingredients in these mushrooms, which possess several pharmacological properties including immune modulation and anti-oxidative effects (He et al. 2017; Li et al. 2021).

Following the 'Mycodigest' prebiotic intervention, we observed an increase in the abundance of two short-chain fatty acid (SCFA) producing bacteria (Ezeji et al. 2021; Shin et al. 2023), *P. distasonis* and *F. umbilicata*. SCFAs are known to promote gut health *via* their immune-modulating and anti-inflammatory effects (Caetano and Castelucci 2022). The potential anti-inflammatory effect of *P. distasonis* has been shown in several animal studies. In a study by Kverka et al. oral administration of *P. distasonis* antigens, attenuated DSS murine colitis model through modulation of immunity and microbiota composition (Kverka et al. 2011). Additionally, a study by Cuffaro et al. showed that in a TNBS induced colitis mouse model, several strains of *P. distasonis* led to gut barrier enhancement and promotion of regulatory T-cell differentiation (Cuffaro et al. 2020). The potential gut beneficial effect of *F. umbilicata* has been shown in the study by Shin et al. in which increased abundance of *F. umbilicata w*as associated with clinical response in subjects with diarrhea-predominant irritable bowel syndrome following multi-strain probiotics supplementation (Shin et al. 2022).

Lastly, we identified an increase in two microbial metabolic pathways related to isoprenoid compounds metabolism, i.e. PWY6859: all-trans-farnesol biosynthesis and polyisoprenoid biosynthesis, following the 'Mycodigest' prebiotic supplementation. Interestingly, farnesol (also known as isoprenoid) has been described as a quorum sensing molecule in fungi (Wang et al. 2017), and has been demonstrated to carry anti-oxidant and anti-inflammatory properties (Ku and Lin 2015; Wang et al. 2017). Farnesol is a type of terpene alcohol predominantly found in essential oils of various plants in nature (Jung et al. 2018). A study by Wang et al. showed that exposure of fungal cultures to farnesol led to enhanced polysaccharide production in *T. versicolor via* promotion of polysaccharide biosynthesis and regulation of fungal morphology resulting in increased antioxidant activity (Wang et al. 2017). Moreover, a mouse study by Ku et al. showed that farnesol supplementation resulted in decreased TNF-$\alpha$ secretion from peritoneal macrophages, highlighting its potential anti-inflammatory effect (Ku and Lin 2015).

Our study has several limitations. First, the sample size in this study was small and therefore our findings will need to be further validated in larger human studies in western population. Second, as the 'Mycodigest' prebiotic used in our study contains three types of mushroom extracts it is hard to determine whether our findings of favorable clinical response and microbial alterations were driven by specific component or by a synergistic effect. Third, although the placebo capsules contained only a small

amount of powder concentrate of Reishi mushroom, it remains to be determined whether it had a clinical or microbial effect beyond its purpose to achieve similar smell to that of the 'Mycodigest' supplement.

In summary, in this pilot study, we observed a modest clinical response to a mushroom based prebiotic intervention in subjects with Crohn's disease, which may be related to favorable compositional and functional microbial alterations.

## Author contributions

NM conceived the study and with HL and NFI designed the study. LW and HL managed and monitored data. HL and NM analyzed and reviewed data, interpreted the results, and drafted the report. NM, NFI, TT, AH, and NAC recruited patients and provided the data. HL, NM, and NAC critically reviewed the manuscript. All authors read and approved the final version of the report.

## Disclosure statement

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## Data availability statement

The data that support the findings of this study are available on request from the corresponding author, [NM].

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