

Saccharomyces boulardii Administration Changes Gut Microbiota and Reduces Hepatic Steatosis, Low-Grade Inflammation, and Fat Mass in Obese and Type 2 Diabetic *db/db* Mice

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ABSTRACT Growing evidence shows that gut microbes are key factors involved in the regulation of energy homeostasis, metabolic inflammation, lipid metabolism, and glucose metabolism. Therefore, gut microbiota modulations caused by selectively fermented oligosaccharides or probiotic bacteria constitute an interesting target in the physiopathology of obesity. However, to date, no probiotic yeast has been investigated in this context. Therefore, our study aimed to evaluate the impact of the most-studied probiotic yeast (i.e., *Saccharomyces boulardii* Biocodex) on obesity and associated metabolic features, such as fat mass development, hepatic steatosis, and low-grade inflammation, in obese mice. *S. boulardii* was administered daily by oral gavage to leptin-resistant obese and type 2 diabetic mice (*db/db*) for 4 weeks. We found that *S. boulardii*-treated mice exhibited reduced body weight, fat mass, hepatic steatosis, and inflammatory tone. Interestingly, these effects of *S. boulardii* on host metabolism were associated with local effects in the intestine. *S. boulardii* increased cecum weight and cecum tissue weight but also induced dramatic changes in the gut microbial composition at the phylum, family, and genus levels. These gut microbiota changes in response to *S. boulardii* may also be correlated with the host metabolism response. In conclusion, this study demonstrates for the first time that *S. boulardii* may act as a beneficial probiotic treatment in the context of obesity and type 2 diabetes.

IMPORTANCE To date, no probiotic yeast have been investigated in the context of obesity and type 2 diabetes. Here we found that type 2 diabetic and obese mice (*db/db*) treated with *Saccharomyces boulardii* exhibited reduced body weight, fat mass, hepatic steatosis, and inflammatory tone. These effects on host metabolism were associated with local effects in the intestine. Importantly, by using pyrosequencing, we found that *S. boulardii* treatment induces changes of the gut microbiota composition at the phylum, family, and genus levels. Moreover, we found that gut microbiota changes in response to *S. boulardii* were correlated with several host metabolism responses.

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Growing evidence supports that gut microbiota-host interactions control energy homeostasis, glucose metabolism, and lipid metabolism (1–4). We and others have shown that the gut microbiota influences whole-body metabolism by affecting energy balance (1–3) and metabolic inflammation associated with obesity and related disorders (5, 6). However, the exact roles of specific microorganisms present in the gut remain poorly defined. Among the different strategies available to modify the gut microbiota in the context of obesity and type 2 diabetes, compelling evidence suggests that oral supplementation with selectively fermented oligosaccharides (i.e., prebiotics, arabinoxylans, and resistant starches) improves these metabolic disorders via several mechanisms (7–12). Moreover, the use of probiotic bacteria has also been suggested (3, 13–19). Strikingly, to our knowledge, the role of probiotic yeast in the modulation of obesity and associated related disorders has never been investigated. The most-studied probiotic yeast is *Saccharomyces cerevisiae* var. *boulardii* Biocodex

(*S. boulardii*), and this yeast has been widely investigated and used for the prevention of antibiotic-associated diarrhea (20). *S. boulardii* differs from other strains by several physiological and metabolic characteristics. For instance, the optimum growth temperature of *S. boulardii* is approximately 37°C, and other strains of *S. cerevisiae* prefer lower temperatures (between 30 and 33°C) for growth (21). In addition, *S. boulardii* is resistant to low pH and is highly tolerant to bile acids (22). *S. boulardii* has been widely characterized, and its beneficial roles have been associated with specific mechanisms, such as specific antitoxin effects, antimicrobial activities, a trophic effect on the gut mucosa, an improved immune response (20), and increased production of butyrate (23), which is a short-chain fatty acid (SCFA) known for its impact on intestinal functions (24).

Although it is well established that *S. boulardii* improves gut health, the potential roles of *S. boulardii* in obesity, associated hepatic disorders, and metabolic inflammation are unknown.

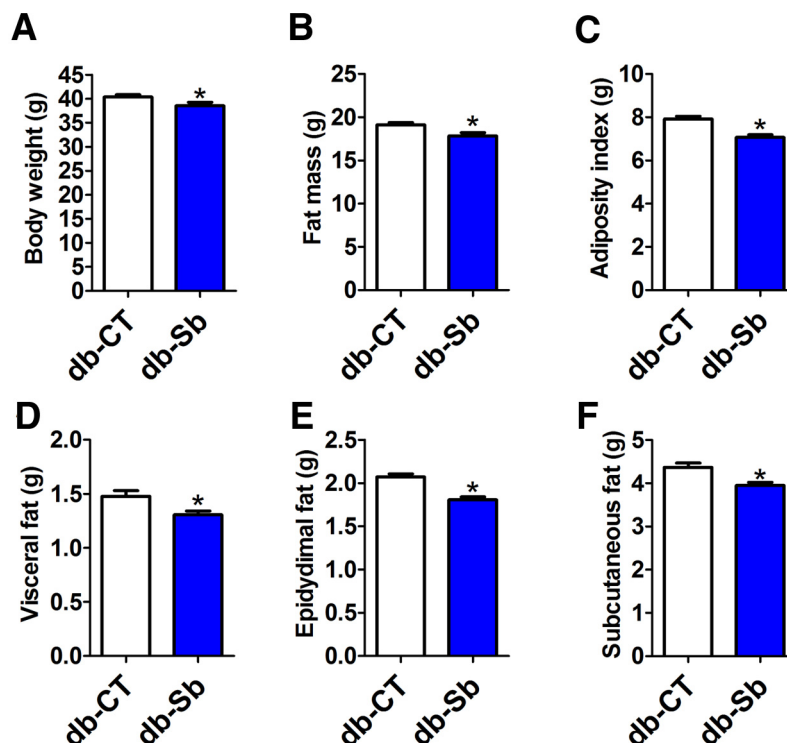


FIG 1 *S. boulardii* administration reduces body weight gain and fat mass in obese and type 2 diabetic mice. Body weight (a), fat mass measured by nuclear magnetic resonance (b), the adiposity index (c), visceral adipose tissue weight (d), epididymal adipose tissue weight (e), and subcutaneous adipose tissue (f) were measured in *db/db* mice treated with the vehicle (saline; db-CT; $n = 15$) or *S. boulardii* (db-Sb; $n = 15$). Data are means $\pm$ SEM. *, $P < 0.05$ according to Student's t test.

Thus, this study had the following aims: (i) to elucidate the impact of *S. boulardii* on obesity, fat mass development, hepatic steatosis, and low-grade inflammation in leptin-resistant obese and type 2 diabetic mice (*db/db*) and (ii) to investigate the influence of *S. boulardii* treatment on the taxonomic profile of the mouse gut microbiota by using high-throughput sequencing analysis.

RESULTS

***S. boulardii* administration reduces body weight gain and fat mass in obese and type 2 diabetic mice.** Leptin-resistant obese and type 2 (*ic>db/db*) diabetic mice develop fatty livers associated with severe obesity and type 2 diabetes (25). Here, we found that after 4 weeks of daily oral gavage with *S. boulardii*, treated (db-Sb) mice exhibited a modest but significant decrease in body weight (Fig. 1a) compared to vehicle-treated mice (db-CT). The body weight gain was about 15% lower in *S. boulardii*-treated animals (10.13 ± 0.56 g in db-CT mice versus 8.71 ± 0.98 g in db-Sb mice [means $\pm$ standard errors of the means, or SEM]; $P > 0.05$). This effect was accompanied by a significantly reduced whole-body fat mass (Fig. 1b) and adiposity index (Fig. 1c), which was assessed by weighing the main fat depots (visceral, epididymal, and subcutaneous) (Fig. 1d to f). This effect was not associated with any changes in food intake (cumulative food intake per mouse. 140.97 ± 6.01 g in db-CT mice versus 149.02 ± 3.81 g in db-Sb mice; $P > 0.05$).

***S. boulardii* administration reduces hepatic steatosis in obese and type 2 diabetic mice.** We found that *S. boulardii* signif-

icantly reduced liver weight (Fig. 2a). To identify if this decrease might be attributed to the fat content, total lipids were extracted from the liver. We found that *S. boulardii* significantly decreased total hepatic lipid content in db-Sb mice compared to db-CT mice (Fig. 2b). These effects were not associated with changes in fasted glycemia (487 ± 22 mg/dl in db-CT versus 489 ± 18 mg/dl in db-Sb; $P > 0.05$) and fasted insulinemia (8.7 ± 0.9 μ g/liter in db-CT versus 7.5 ± 0.8 μ g/liter in db-Sb; $P > 0.05$).

***S. boulardii* administration decreases hepatic and systemic inflammation.** Evidence suggests that obesity is associated with the development of inflammatory liver diseases, such as nonalcoholic fatty liver disease and nonalcoholic steatohepatitis (26, 27). We have also previously demonstrated that the gut microbiota contributes to the development of hepatic steatosis and inflammation (7, 28, 29). In the present study, we found that the decreased hepatic steatosis observed in db-Sb mice was associated with a marked decrease in liver macrophage infiltration markers, as shown by the 50% decrease in cluster of differentiation 11c (CD11c) and F4/80 mRNA levels as well as the reduced expression (to approximately 40%) of monocyte chemoattractant protein 1 (MCP-1) mRNA (Fig. 2c to e). In accordance with the lower expression of macrophage infiltration markers, we found that *S. boulardii* treatment reduced liver interleukin-1 β (IL-1 β) mRNA levels by approximately 37% compared to vehicle-treated mice (Fig. 2f). In addition to the reduced hepatic inflammation, we found that systemic markers of inflammation were reduced following *S. boulardii* treatment. Plasma cytokine concentrations of IL-6 and IL-4 were significantly reduced by approximately

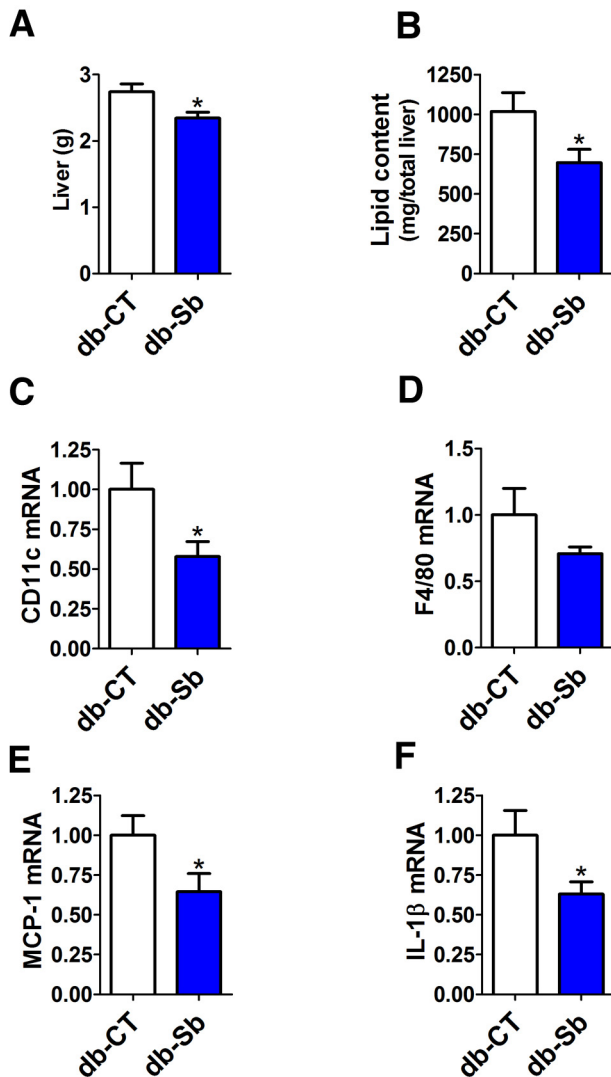


FIG 2 *S. boulardii* administration reduces liver weight, hepatic steatosis, and hepatic inflammatory markers in obese and type 2 diabetic mice. Liver weight (a), total lipid liver content (b), and inflammatory marker mRNA expression of CD11c (c), F4/80 (d), MCP-1 (e), and IL-1β (f) measured in *db/db* mice treated with the vehicle (saline; db-CT; *n* = 15) or *S. boulardii* (db-Sb; *n* = 15). mRNA data are expressed relative to results in db-CT mice. Data are means ± SEM. *, *P* < 0.05 according to Student's *t* test.

2-fold (Fig. 3a and c) in db-Sb mice compared to db-CT mice. IL-1β was reduced by approximately 40% (*P* = 0.06), and tumor necrosis factor alpha (TNF-α) was reduced by approximately 20% (*P* = 0.12) (Fig. 3b and d).

***S. boulardii* significantly increases cecum weight.** The gut mucosa is subjected to a constant and rapid cellular turnover (30, 31), and *S. boulardii* has been shown to exert a trophic effect on gut mucosa (32, 33). Here we found that *S. boulardii* significantly increased cecum weight and cecal tissue weight, thereby suggesting a trophic effect on this tissue (Fig. 4a and b).

***S. boulardii* profoundly affects the gut microbial community at different taxonomic levels.** We first quantified the abundance of total yeast cells as well as total *Saccharomyces* cells. The number of yeast cells reached $6.57 \pm 0.09 \log_{10}$ cells/g of cecal content in

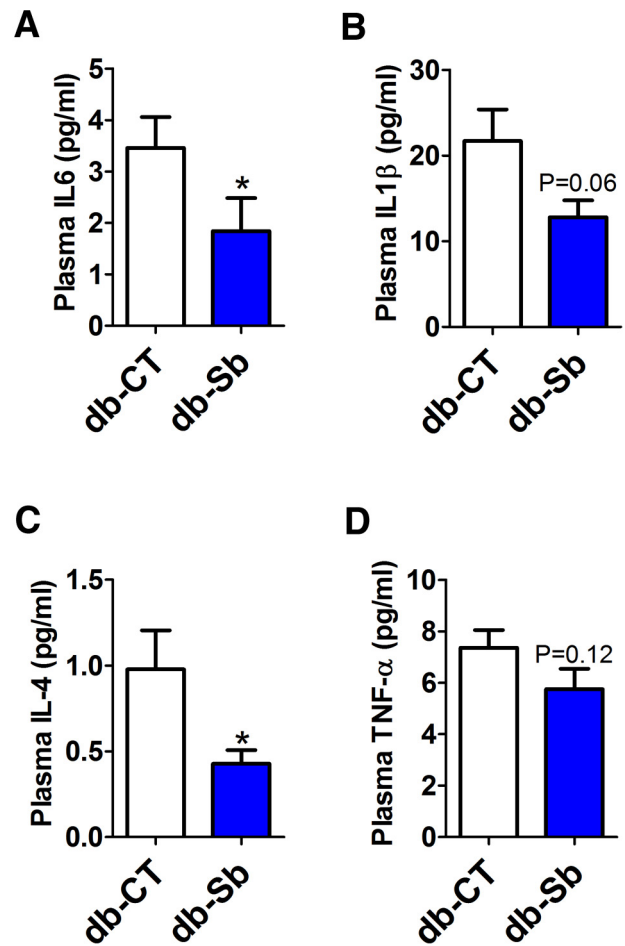


FIG 3 *S. boulardii* administration reduces plasma cytokines in obese and type 2 diabetic mice. Plasma cytokine concentrations of IL-6 (a), IL-1β (b), IL-4 (c), and TNF-α (d) measured in the plasma of *db/db* mice treated with the vehicle (saline; db-CT; *n* = 15) or *S. boulardii* (db-Sb; *n* = 15). Data are means ± SEM. *, *P* < 0.05 according to Student's *t* test.

db-CT mice and $8.21 \pm 0.17 \log_{10}$ cells/g of cecal in db-Sb mice; *P* = 1×10^{-15}). The abundance of total *Saccharomyces* was $5.85 \pm 0.09 \log_{10}$ cells/g of cecal content in db-CT mice and $8.08 \pm 0.22 \log_{10}$ cells/g of cecal content in db-Sb mice (*P* = 5.9×10^{-17}), thereby showing that *S. boulardii* administration increased by about 2 logs the abundance of *Saccharomyces* yeast cells in the cecal content of mice. Principal coordinates analysis (PCoA) showed that the overall gut microbial community was significantly modified by *S. boulardii* treatment (Fig. 5a). We previously demonstrated that *db/db* mice present an altered gut microbiota composition that is characterized by a decrease in the abundance of the phylum *Bacteroidetes*, an increase of *Firmicutes*, and a dramatic increase in *Proteobacteria* compared to lean mice (25). In the present study, we found that *S. boulardii* treatment profoundly affected the abundance of different phyla. For instance, we found that *S. boulardii* was associated with a significant increase in *Bacteroidetes* (by approximately 37%) and a significant decrease in the abundance of *Firmicutes* (by 30%) compared to db-CT mice (Fig. 5b; see also Table S2 in the supplemental material). Both *Proteobacteria* and *Tenericutes* were profoundly affected by the

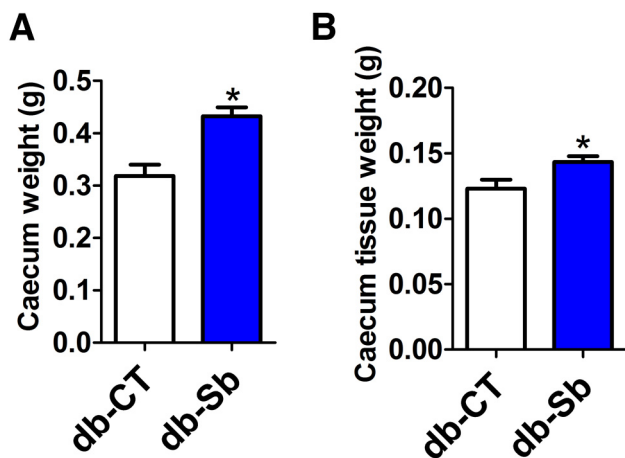


FIG 4 *S. boulardii* administration increases cecum weight and cecum tissue weight in obese and type 2 diabetic mice. Total cecum weight (a) and cecum tissue weight (b) were measured in *db/db* mice treated with the vehicle (saline) db-CT; *n* = 15) or *S. boulardii* (db-Sb; *n* = 15). Data are means $\pm$ SEM. *, *P* < 0.05 according to Student's *t* test.

treatment, as we found decreases of 55 and 57%, respectively. These results suggested that *S. boulardii* changes the gut microbial community by affecting the relative fractional abundance of the four main phyla (Fig. 5b; see also Table S2). At the family level, we found several important modifications of the gut microbiota composition. Among the 34 families identified, 5 of them were significantly changed following *S. boulardii* treatment, after correction via a false-discovery rate (FDR) test according to the Benjamini-Hochberg procedure (Fig. 5c; see also Table S3 in the supplemental material). The major differences were observed at the level of the dominant families, as follows: the *Bacteroidaceae* family was increased by 6-fold in db-Sb mice, and *Porphyromonadaceae* was decreased by 8-fold in db-Sb mice (Fig. 5c; see also Table S3).

Among the 30 genera detected, *Bacteroides* was the most abundant, with a mean abundance of 8.3% across all samples. In the *S. boulardii* treatment group, this genus was increased by 400%. Conversely, the following genera were decreased in *S. boulardii*-treated mice: *Anaeroplasma* (−92%), *Anaerotruncus* (−47%) *Dorea* (−77%), *Odoribacter* (−82%), *Oscillospira* (−38%), *Parabacteroides* (−91%), *Prevotella* (−76%), and *Ruminococcus* (−44%) (Fig. 5d; see also Table S4 in the supplemental material).

***S. boulardii*-induced modifications of the gut microbiota correlates with metabolic parameters.** We performed a Spearman correlation analysis corrected by a false-discovery rate test according to the Benjamini-Hochberg procedure in order to evaluate the potential link between significant changes in gut microbiota composition induced by *S. boulardii* and host metabolism (Fig. 6).

We found that several markers were positively or negatively associated with body weight, fat mass, cecum weight, hepatic steatosis, or inflammatory markers. For instance, we found that the adiposity index and specific adipose tissue weights were significantly associated with several genera. *Odoribacter*, *Parabacteroides*, *Prevotella*, and *Ruminococcus* were all positively associated with the adiposity index or epididymal adipose tissue (EAT) weight, whereas *Bacteroides* was inversely associated with fat mass

(Fig. 6). We found that cecum weight was negatively correlated with *Prevotella*, and *Anaerotruncus*. Among the different genera affected by *S. boulardii*, we found that *Bilophila* was the only genus that was negatively correlated with liver tissue weight.

In contrast, *Ruminococcus* was positively associated with these parameters and with F4/80 mRNA expression (Fig. 6).

DISCUSSION

This study demonstrated that *S. boulardii* administration in obese and type 2 diabetic mice profoundly modifies host metabolism and is associated with changes in the gut microbial composition. *S. boulardii*-treated mice exhibited reduced fat mass, hepatic steatosis, and inflammatory tone, thereby suggesting that *S. boulardii* may also act as a beneficial probiotic treatment in the context of obesity and type 2 diabetes. To our knowledge, this study is the first high-throughput study that has analyzed the effects of this yeast on the gut microbiota as well as the first study that has shown an impact of *S. boulardii* on metabolic disorders associated with cardiometabolic risk factors, such as fat mass development, steatosis, and inflammation. Nevertheless, numerous studies have already shown a protective effect of *S. boulardii* in different models associated with inflammation (e.g., inflammatory bowel diseases, colitis, intestinal infections, and hepatic injury) (20, 34–36). We did not find any changes in food intake between groups. This observation suggested that *S. boulardii* modulates energy homeostasis via a mechanism other than energy intake. Importantly, we found that *S. boulardii* treatment reduced hepatic and systemic inflammation. Because liver lipid accumulation is associated with liver and systemic inflammation, one may postulate that the decreased inflammatory tone may be related to the lower liver and whole-body fat accumulation. However, the impacts of *S. boulardii* on both fat mass and body weight were approximately 10%, and the inflammatory markers were reduced by 40 to 50%. Therefore, we suggest that *S. boulardii* contributed to the reduced inflammation by a putative gut-to-liver axis. Given that this treatment has been previously associated with an improved gut barrier function (20, 32, 34–38), we may not exclude that *S. boulardii* improved the gut barrier function in this model. Regarding intestinal integrity, we found that *S. boulardii* treatment increased both cecum and cecal tissue weights, thereby suggesting a trophic effect of the yeast on the intestinal epithelial cells. These results were also consistent with previous studies that showed that *S. boulardii* exerts a trophic effect on the intestinal epithelium via several molecular mechanisms (33, 37).

We and others previously demonstrated that the gut microbiota contributes to the development of hepatic steatosis, hepatic inflammation, and systemic inflammation, but the impact of *S. boulardii* on the gut microbiota is poorly defined (7, 28, 39–43). Thus, we decided to determine the impact of *S. boulardii* on the abundance of total yeast and the genus *Saccharomyces* but also on the gut microbiota composition by using a high-throughput sequencing method. We found that *S. boulardii* administration increased by about 160-fold the abundance of total *Saccharomyces* in the cecal content, whereas the total number of yeast cells was increased by about 40-fold, thereby increasing the relative proportion of *Saccharomyces* cells per total yeast cells from 18.9% to 73.9%. Thus, this result suggests that the relatively lower increase in total yeast cells observed upon *S. boulardii* treatment might be explained by a modification of the abundance of other yeasts. However, this hypothesis merits further investigation.

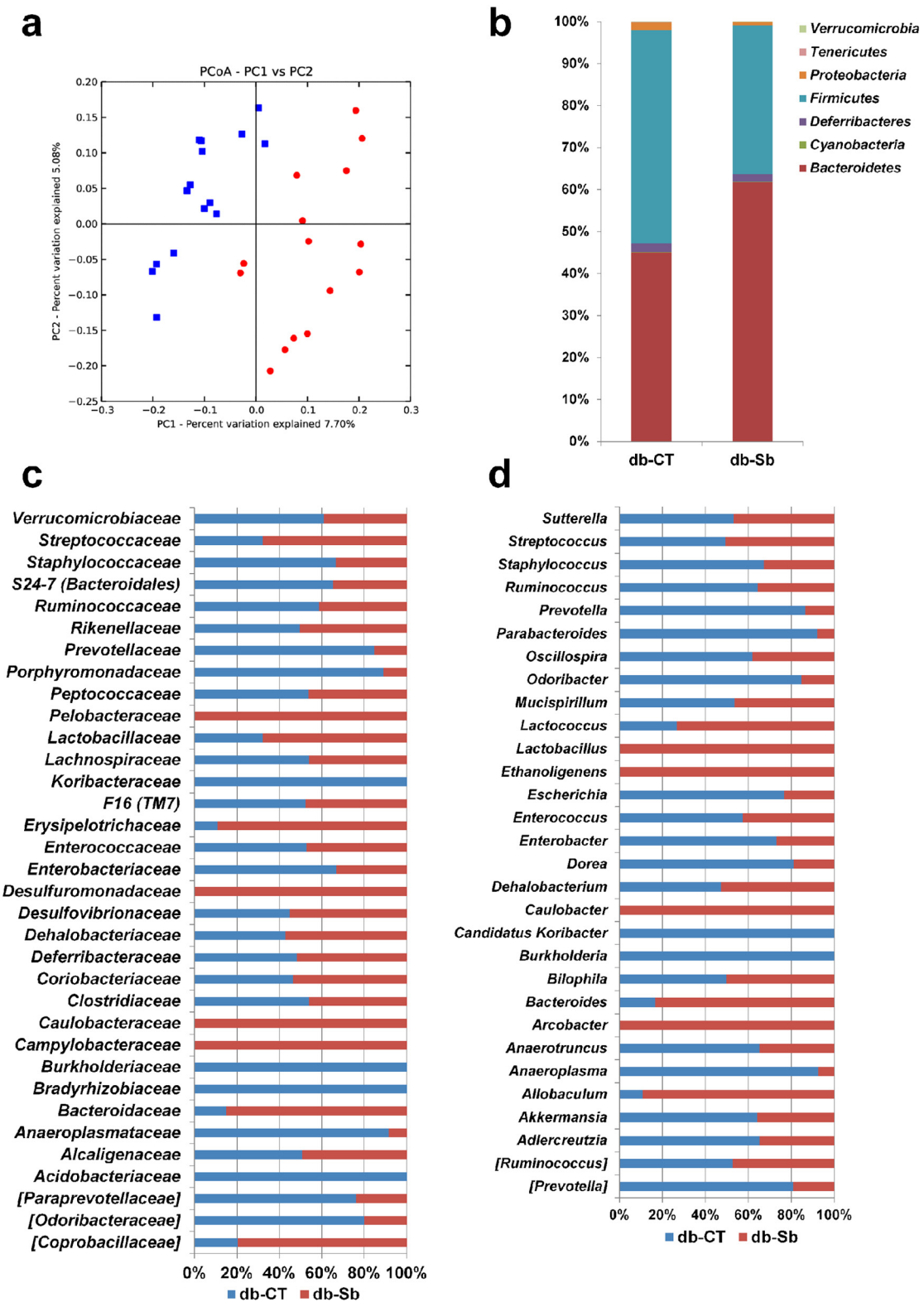


FIG 5 *S. boulardii* administration changes the gut microbiota composition at different taxonomic levels. (a) PCoA results for the gut bacterial community, based on the weighted Unifrac analysis of the different OTUs in db-CT (red dots) and db-Sb (blue squares) mice. Phyla (b), families (c), and genera (d) were detected in the cecal contents of *db/db* mice treated with the vehicle (saline; db-CT; $n = 15$) or *S. boulardii* (db-Sb; $n = 15$). Undetected taxa are not represented in the graphic. In panels c and d, each column is set at 100% to illustrate the proportion of each taxa among the two groups; the presence of only one color indicates that the taxa was present only in this group of mice. The significant changes and raw values of each taxa are shown in the supplemental material.

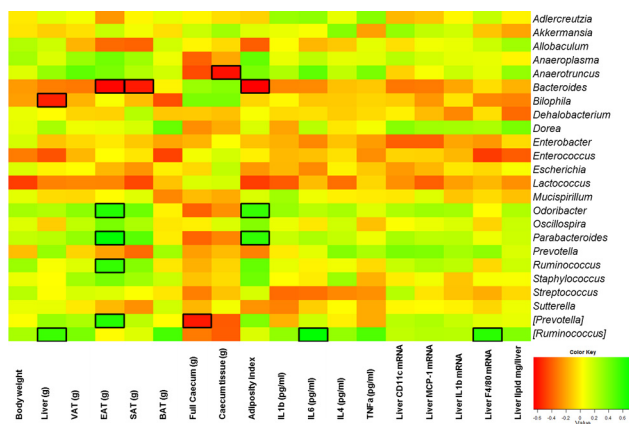


FIG 6 Specific genera are correlated with several host markers. The heat map shows the Spearman r correlations between the bacterial genera detected in the cecal contents of *db/db* mice treated with the vehicle (saline; db-CT; $n = 15$) or *S. boulardii* (db-Sb; $n = 15$). Square cells depict significant differences in treated and control animals following a Spearman correlation analysis. *, $P < 0.05$. BAT, brown adipose tissue; EAT, epididymal adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue weight.

Here, we found that *S. boulardii* significantly changed the gut microbiota composition with an increased proportion of *Bacteroidetes* and a decreased amount of the phyla *Firmicutes*, *Proteobacteria*, and *Tenericutes*. These phyla have been previously associated with obesity and type 2 diabetes in mice, with a higher abundance of *Firmicutes*, *Proteobacteria*, and *Tenericutes* as well as a lower abundance of *Bacteroidetes* (8, 25, 44–46). Moreover, we found that *S. boulardii* treatment affected several genera that have been previously associated with diabetes and inflammation in *db/db* mice (i.e., *Odoribacter*, *Ruminococcus*, and *Prevotella*) (25). Thus, we speculate that, in response to *S. boulardii*, the gut microbiota may contribute to the host metabolism response. However, the relationships that exist between *S. boulardii* and specific microbes remain unknown. *S. boulardii* has been shown to modify the production of SCFAs, such as butyrate (23). Evidence suggests that butyrate may contribute to the regulation of several functions at the level of the gut barrier but also to energy homeostasis (47–49) and hepatic steatosis (50). Further investigation is required to understand whether the positive effects observed upon *S. boulardii* treatment are mediated through butyrate- or SCFA-dependent mechanisms. In the present study, we found that 7 families among the 34 identified were significantly affected by the treatment. At the genera level, 9 of the 30 genera identified were affected by *S. boulardii* treatment. Importantly, most of them are poorly characterized and could be novel bacteria to study in the future in the context of obesity, because we cannot rule out that these specific changes in genera are involved in the beneficial effects of *S. boulardii* on host metabolism. Because some of the genera affected by *S. boulardii* are correlated with metabolic parameters, we postulate that these specific changes in the gut microbiota may contribute to the beneficial effects of *S. boulardii* on host metabolism. However, whether these genera directly contribute to the phenotype warrants further investigation. For instance, we found a decrease in *Prevotella* in *S. boulardii*-treated mice and a positive correlation between *Prevotella* and adipose tissue weight (EAT). These data were in accordance with data reported elsewhere in the literature, because Zhu et al. found an increase in these bacteria in

human obese and nonalcoholic steatohepatitis patients, thereby suggesting a link between the presence of these bacteria and fat mass (51).

Interestingly, our results showed that *Bacteroides* was dramatically increased by *S. boulardii* treatment. Moreover, we found an inverse correlation between *Bacteroides* and the fat mass, suggesting a potential beneficial effect of this bacterium in host physiology. This result was in accordance with our recent study that showed that a prebiotic-enriched diet is associated with an increase in *Bacteroides* compared to a high-fat diet (52), and this result was also in accordance with a second study that revealed that treatment with alkaloid berberine, a plant that prevents obesity, is associated with an increase in this genus (53). In addition to its probiotic effect and immunomodulatory properties, *S. boulardii* could act as a prebiotic, which would explain the impressive increase of the *Bacteroides* genus in db-Sb mice. The cell walls of yeasts are made up of various proportions of β -glucans (54). These polysaccharides are poorly digested by the host due to a lack of specific enzymatic tools necessary for their digestion, but they can be fermented by intestinal bacteria (55). The genus *Bacteroides* has been recognized for a long time for its ability to metabolize this particular class of polysaccharides and could benefit greatly from an additional presence of this compound in the intestine for growth (56–58). Whether this specific effect on *Bacteroides* is restricted to the strain *S. boulardii* requires further investigation.

In conclusion, our results demonstrated that *S. boulardii* intervention in mice may change the gut microbiota and reduce fat mass, hepatic steatosis, systemic inflammation, and hepatic inflammation in obese and type 2 diabetic mice. We identified a novel potential therapeutic role of *S. boulardii* treatment that profoundly affects numerous host metabolic parameters. Moreover, this is the first study that has provided a deep analysis of the gut microbiota modulations that occur after *S. boulardii* supplementation. In addition, we observed putative correlations between genera and several metabolic markers. Thus, our results provide new insights into the complex relationships that exist between *S. boulardii* yeast and several taxa on metabolism in the context of metabolic inflammation and obesity.

MATERIALS AND METHODS

Mice and treatment. A set of 6-week-old *db/db* mice ($n = 15$ /group) (BKS.Cg-Dock^{7m}+/+*Lep^{db}*); Jackson Laboratory, Bar Harbor, ME) were housed in a controlled environment (12-h daylight cycle; lights off at 6 p.m.) in groups of two or three mice/cage. The mice were fed a control diet (CT; AIN93M; Research Diet, New Brunswick, NJ). *Saccharomyces boulardii* was provided by Biocodex (France). *S. boulardii* was suspended in sterile saline and immediately administered by oral gavage (120 mg; db-Sb), and the control group (db-CT) received the same volume of sterile saline solution. The treatment was continued for 4 weeks. Body composition was assessed by using a 7.5-MHz time domain nuclear magnetic resonance (TD-NMR) apparatus (LF50 Minispec; Bruker, Rheinstetten, Germany). The experiment was approved by and performed in accordance with the guidelines of the local ethics committee. Housing conditions were specified by the Belgian Law of 29 May 2013 regarding the protection of laboratory animals (agreement number LA1230314).

Tissue sampling. The animals were anesthetized with isoflurane (Isoba; Schering-Plough Animal Health, Uxbridge, Middlesex, United Kingdom) before exsanguination and tissue sampling, and the mice were then killed by cervical dislocation. Visceral (corresponding to the mesenteric fat), brown, epididymal, and subcutaneous (corresponding to the inguinal and fat pads located on the lower back) adipose tissues were precisely dissected and weighed. The adiposity index corresponds to the

sum of the different adipose tissue weights (visceral, epididymal, subcutaneous, and brown). Liver tissue was weighed, snap-frozen in liquid nitrogen, and stored at -80°C until further analysis. The cecum was weighed, and the cecal content was collected for microbiota analyses, immersed in liquid nitrogen, and stored at -80°C until further analysis. Cecal tissue was washed in cold saline, dried, and weighed.

Plasma cytokine measurement. Plasma contents of IL-1, IL-6, IL-4, and TNF- α were determined in duplicate by using Bio-Plex Pro cytokine assays kit (Bio-Rad, Nazareth, Belgium) and measured using a Luminex instrument (Bio-Plex; Bio-Rad) following the manufacturer's instructions.

RNA preparation and real-time qPCR analysis. Total RNA was prepared from tissues by using TriPure reagent (Roche). Quantification and integrity analysis of total RNA were performed by analyzing 1 μl of each sample in an Agilent 2100 bioanalyzer (RNA 6000 Nano kit). cDNA was prepared by reverse transcription of 1 μg of total RNA by using a reverse transcription system kit (Promega, Leiden, The Netherlands). Real-time PCR was performed with the StepOnePlus real-time PCR system and software (Applied Biosystems, Den IJssel, The Netherlands) and Mesa Fast quantitative PCR (qPCR; Eurogentec, Seraing, Belgium) for detection according to the manufacturers' instructions. RPL19 RNA was chosen as the housekeeping gene. All samples were performed in duplicate in a single 96-well reaction plate, and data were analyzed according to the $2^{-\Delta\text{CT}}$ method. The identity and purity of the amplified product were assessed by melting curve analysis at the end of amplification. The primer sequences for the targeted mouse genes are presented in Table S1 in the supplemental material.

Liver lipid content. Total lipids were measured in the liver tissue after an extraction in CHCl_3 -methanol (MeOH) according to the method of Folch et al. (59), adapted as previously described (60). Briefly, 100 mg of liver tissue was homogenized in 1 ml of phosphate buffer (pH 7.4) by using an Ultra-Turrax instrument (IKA, T10 basic; IMLAB, Boutersem, Belgium) until complete tissue lysis. Lipids were extracted by mixing 125 μl of lysate sample with 1 ml of CHCl_3 -MeOH (2:1). The chloroform phase was evaporated under nitrogen flux, and the dried residue was weighed to determine the total lipid content.

DNA isolation from mouse cecal samples. Metagenomic DNA was extracted from the cecal contents by using a QIAamp-DNA stool minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and the adapted procedure previously described (61). The quantity and the quality of the DNA extracted from the samples were checked before sending the samples for sequencing.

Sequencing. The 16S rRNA gene from the cecal microbiota of the mice was amplified using the universal Eubacterial primers as follows: 27Fmod (5' AGRGTTTGATCMTGGCTCAG 3') and 519Rmodbio (5'-GTNTTACNGCGGCKGCTG-3'). Purified amplicons were sequenced utilizing Roche 454 FLX titanium instruments and reagents following the manufacturer's guidelines.

Sequencing was performed at MR DNA (Shallowater, TX).

The Q25 sequence data derived from the sequencing process were analyzed with the QIIME 1.7 pipeline. In summary, sequences were depleted of bar codes and primers. Sequences of <200 bp or >1,000 bp were then removed, and sequences with ambiguous base calls and with homopolymer runs exceeding 6 bp were also removed. Sequences were denoised, and operational taxonomic units (OTUs) were generated. Moreover, chimeras were removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated GreenGenes database. PCoA was generated with QIIME using the unweighted UniFrac distance matrix between the samples (62, 63).

Yeast cell quantification. Yeast cells were quantified using the primers YEASTF (5' GAGTCGAGTTGTTTGGGAATGC 3') and YEASTR (5' TCTCTTCCAAAGTTCTTTTCATCTTT 3') following the method described by Hierro et al. (64). *Saccharomyces* was quantified using primers SC1 (5' GAAACTCCACAGTGTGTTG 3') and SC2 (5' GCTTAAGTG

CGCGGTCTTG 3') according to the method described by Zott et al. (65). Detection was achieved with the StepOnePlus real-time PCR system and software (Applied Biosystems) and Mesa Fast qPCR (Eurogentec) according to the manufacturer's instructions. Each assay was performed in duplicate in the same run. The cycle threshold (C_T) of each sample was then compared with a standard curve (performed in triplicate) made by diluting genomic DNA (5-fold serial dilution) extracted from a pure culture of *S. boulardii* (Biocodex). The data are expressed as the $\log_{10}$ of bacteria per g of cecal content.

Statistical analysis. Data are expressed as means $\pm$ SEM unless otherwise indicated. Differences between two groups were assessed using the unpaired two-tailed Student's *t* test. Data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Data related to the gut microbiota were analyzed using JMP 8.0.1 (SAS Institute, Inc., Cary, NC) and R 3.0.2 (The R Foundation) with the RStudio 0.97.310 package and gplots for the heat map. The results were considered statistically significant at *P* level of <0.05 . Correlation results were corrected by an FDR test according to the Benjamini-Hochberg procedure, with an α of <0.05 .

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01011-14/-DCSupplemental>.

Table S1, DOC file, 0.1 MB.

Table S2, DOCX file, 0.1 MB.

Table S3, DOCX file, 0.1 MB.

Table S4, DOCX file, 0.1 MB.

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