REVIEW

Saccharomyces boulardii CNCM I-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis – a review

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Abstract: The probiotic medicinal yeast *Saccharomyces cerevisiae* HANSEN CBS 5926 (*Saccharomyces boulardii* CNCM I-745) is used for the prevention and treatment of diarrhea. Its action is based on multiple mechanisms, including immunological effects, pathogen-binding and antitoxinic effects, as well as effects on digestive enzymes. Correlated with these effects, but also due to its inherent properties, *S. boulardii* is able to create a favorable growth environment for the beneficial intestinal microbiota, while constituting extra protection to the host mucus layer and mucosa. This review focuses on the positive influence of *S. boulardii* on the composition of the intestinal microbiota. In a dysbiosis, as during diarrhea, the main microbial population (especially Lachnospiraceae, Ruminococcaceae, Bacteroidaceae, and Prevotellaceae) is known to collapse by at least one order of magnitude. This gap generally leads to transient increases in pioneer-type bacteria (Enterobacteriaceae, Bifidobacteriaceae, and Clostridiaceae). Several human studies as well as animal models demonstrate that treatment with *S. boulardii* in dysbiosis leads to the faster reestablishment of a healthy microbiome. The most relevant effects of *S. boulardii* on the fecal composition include an increase of short chain fatty acid-producing bacteria (along with a rise in short chain fatty acids), especially of Lachnospiraceae and Ruminococcaceae, as well as an increase in Bacteroidaceae and Prevotellaceae. At the same time, there is a suppression of pioneer bacteria. The previously observed preventive action of *S. boulardii*, eg, during antibiotic therapy or regarding traveler's diarrhea, can be explained by several mechanisms, including a stabilizing effect on the healthy microbiota as well as possibly on the mucus layer. Several different dysbiotic situations could profit from the effects of *S. boulardii* CNCM I-745. Its additional potential lies in a general stabilization of the gut flora for at-risk populations. More studies are needed to explore the full potential of this versatile probiotic yeast.

Keywords: *Saccharomyces boulardii* CNCM I-745, microbiota, diarrhea, dysbiosis, colonic bioreactor, intestinal mucus layer, mucosa, SCFA, probiotic

Search method

The research was done in the databases Medline (http://www.ncbi.nlm.nih.gov/pubmed) and PubMed Central (PMC; http://www.ncbi.nlm.nih.gov/pmc/), searching for *Saccharomyces boulardii* (480 entries in Medline and 705 entries in PMC as of January 2015). All publications detailing effects of *S. boulardii* on the microbiota were evaluated. Further literature was found after searching for terms relevant to the specific topic (eg, combinations of colon, study, SCFA [short chain fatty acid], butyrate, immune system, microbiota, dysbiosis, antibiotic, probiotic, prebiotic, etc) and following up literature citations.

Introduction

Objective of this review

This review focuses on the positive influence of *S. boulardii* on the composition of the intestinal microbiota. To understand the effects of the probiotic yeast, we first provide an up-to-date review on the function of the colon and its microbiota in the healthy situation as well as during dysbiosis.

We then describe the properties of *S. boulardii*, recapitulate its clinical success, and sum up its mechanisms of action, both in the healthy situation and during dysbiosis. These mechanisms of action result in its effects on the microbiota, which have been observed in various nonclinical and clinical studies. We summarize all nonclinical and clinical studies evaluating the effects of *S. boulardii* on the microbiota, which have been published to date. Derived from the study results, we conclude specific effects of *S. boulardii* on various microbial groups during dysbiosis, leading to an overall improved regeneration of the microbiota. In the final discussion, a broader use potential of *S. boulardii* is suggested.

The healthy colon and its microbiota

In the healthy intestine, a thick mucus layer separates the intestinal wall from the densely populated intestinal contents, with 10^{12} bacteria/g of stool within the colon.[1] The contained bacteria include many potentially harmful species, which thrive on the nutrient supply from the already partially pre-digested food in a mostly fermentative lifestyle.

The colonic "bioreactor" has several important functions:

- The microbial production of SCFA (eg, butyrate), a prominent nutrient for the human host fulfilling various regulatory functions[2–6]
- The microbial production of valuable secondary metabolites and vitamins, which are taken up by the human host – colectomy leads to malnutrition[7]
- Nutrient/drug processing and salvage[8,9]
- General immune system awareness[10–12]
- Influence on energy homeostasis, glucose metabolism, and lipid metabolism[13]
- Water and electrolyte recycling from the intestinal contents.

The bioreactor is kept running by a microorganism-induced program of epithelial cell homeostasis and repair.[14] In addition, the colonic mucus disables bacterial movements and excludes the microbiota from direct epithelial contact.[15]

The mucus consists of mucin glycoproteins, produced by goblet cells.[15] It is condensed by continuous water resorption.

The inner mucin layer is densely packed, and the small pore size physically prevents bacterial penetration.[16]

The outer part of the mucus layer is more porous and is infiltrated with bacteria.[17] It may form laminar mucus structures. This so-called germinal stock area functions as a reservoir of the microbiota, even if most of them are regularly discharged.[18] The feces itself is also covered by mucus, derived from the outer mucus layer.

The colonic microbiota during intestinal dysbiosis

Intestinal dysbiosis can be defined as an unfavorable dysbalance of the intestinal microbiota. In addition, microscopic examination has shown a disruption of the protective mucus layer for different diarrheic dysbiotic situations, including inflammatory bowel disease (IBD; either Crohn's disease or ulcerative colitis), irritable bowel syndrome (IBS), acute diarrhea, human immunodeficiency virus (HIV) enteropathy, and other intestinal conditions, resulting in bacteria directly attaching to the exposed mucosa and eliciting a polymicrobial infection.[15,19,20]

Several conditions may lead to a dysbiosis; in addition, there are certain risk factors, eg, malnutrition, old age, diabetes/metabolic syndrome,[21,22] and stress, that additionally destabilize the microbiota (Figure 1).

The disruption of the normal intestinal microflora by antibiotics is a common cause of dysbiosis in the industrialized world. Individuals who never received antibiotics throughout their lifetime are a minority, a fact that raises concerns since this practice may lead to a long-term deprivation and alteration of the microbiota within the entire population.[23–25]

Both parenteral and enteral nutrition were found to lead to a bacterial unbalance;[26,27] specifically, butyrate producers and starch degraders were found to be drastically reduced in the feces of critically ill tube-fed patients, with promising antidiarrheic results after fiber supplementation.[28,29]

Irritable bowel syndrome is characterized by an increased permeability and an altered immune profile, as well as central nervous and gut neuromuscular impacts.[30] This can lead to small intestinal bacterial overgrowth as well as hypersensitive bowel movements.[31]

The chronic inflammatory conditions, Crohn's disease and ulcerative colitis, go along with a reduced microbial diversity and differences in the microbial community structure at the inflamed sites.[32] For chronic situations as in IBD, the cause–effect relationship may rather be a vicious circle between dysbiosis and altered physiology.[33]
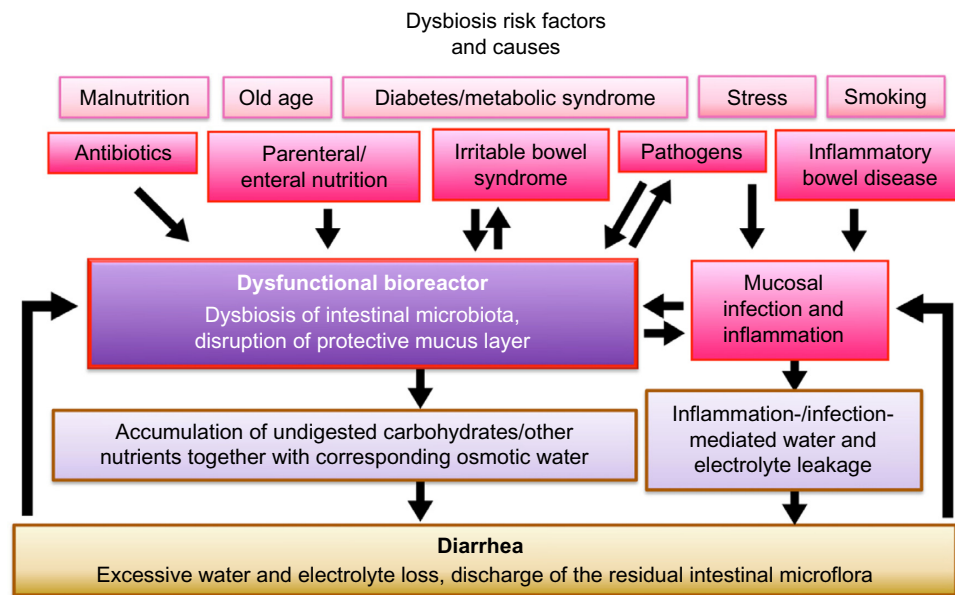
Figure 1 Dysbiosis of intestinal microbiota during diarrhea.
Notes: The gastrointestinal tract harbors a complex microbial ecosystem that coexists in equilibrium with the host. When this equilibrium is disrupted, dysbiosis can manifest itself in a vicious cycle, prolonging diarrheic symptoms.

Certainly, many cases of dysbiosis – including life-threatening diarrheic conditions – are induced by pathogens (eg, rotaviruses or various bacterial pathogens).[34–36] On top of this, acute gastrointestinal infections lead to an increased incidence of IBS.[37] Also, an association between antibiotic use and an increased risk of persistent digestive symptoms in children has been shown.[38] Vice versa, a disruption of the intestinal bacterial population along with a disruption of the mucus barrier favors bacterial pathogens (eg, *Peptoclostridium [Clostridium] difficile*).[39]

In addition, people affected by autism spectrum disorders display symptoms of gastrointestinal disturbance along with an altered microbiota.[40,41] Interestingly, also extreme, life-threatening physical situations, like stroke or severe brain injury, lead to diarrheic dysbiosis, accompanied by leukocyte infiltration of the mucus layer similar to ulcerative colitis, with similar microbial changes.[42]

Dysbiosis along with a mucus destabilization can result in diarrhea and accompanying conditions like bloating, flatulence, and cramping.

This can be explained as follows:

1. A disrupted microflora can only insufficiently bind and metabolize the minerals, carbohydrates, and other nutrients usually taken up as "nutrition" – they remain in the intestinal lumen together with a corresponding quantity of osmotic water: the result is more watery stools.
2. Also, the reduction of SCFA (eg, butyrate)-producing bacteria results in a reduced amount of SCFA, with consequences.

Normally, SCFA signaling in the intestine suppresses insulin-mediated fat accumulation[43] and inhibits food intake by appetite control.[3] Thus, long-term SCFA reduction is correlated with increase in body weight.[22] This goes along with the observation that obese people suffer from chronic diarrhea more often than normal weight people.[44]

In addition, SCFAs have epithelial growth-promoting and anti-inflammatory effects[45] as well as a variety of other health-promoting effects.[5] Thus, if SCFAs are reduced, inflammation and disease can take over more easily.

3. The activation of an immune and repair response in the intestine is induced by bacterial products reaching Toll-like receptors on the inner side of the epithelial cells due to damage of the barrier function.[14]

A mucosal inflammatory response results in a reduced tight junction-mediated barrier function,[46–48] correlated with water and electrolyte leakage. This condition also can be considered an attempted discharge of potential pathogens, together with a substantial number of the other intestinal inhabitants.

4. As a consequence, the total microbial concentration in the colon is significantly reduced during diarrhea.[18,49] This goes along with increased intestinal motility. The bioreactor is purging itself.

Inflammatory reactions like leukocyte infiltration have the role of clearing the mucosa from potential pathogens. Replacement of damaged or infected epithelial cells and

rebuilding of an intact mucus layer are prerequisite for the return to a healthy situation.

Although dysbiosis often leads to diarrhea, there may be situations in which an unbalanced microbiota produces regular stools most of the time, while still manifesting itself in a low-level intestinal inflammation.

Especially in countries with high technology standards, an increasing amount of food containing a high burden of detergents and emulsifiers is consumed. These agents, eg, carboxymethyl cellulose, have been shown to have detrimental effects on the integrity of the intestinal mucus layer, thereby possibly furthering low-level infection – direct attachment to the mucosa – of commensal bacteria.[50]

In addition, there is evidence that constipation is correlated with dysbiosis.[51,52]

Thus, those humans in the industrialized world who are threatened by dysbiosis through risk factors like stress, old age, increased body mass/diabetes/metabolic syndrome, malnutrition, or otherwise unhealthy lifestyles together with those already suffering dysbiotic symptoms may make up a significant proportion of the entire population.

Properties of *S. boulardii* and summary of clinical success and its mechanisms of action
Probiotic properties of *S. boulardii*

The natural healthy gastrointestinal microbiota only has a yeast content of <0.1%, *Candida albicans* being the most prominent yeast inhabitant.[53]

Even taking into account a "correction for the 10 times larger size," yeast represents less than 1% of the total microbiota.[53]

The well-known nonpathogenic medicinal yeast *S. boulardii* was originally isolated in 1923 by Henri Boulard from peels of tropical fruit. Probiotic strains of *S. boulardii* belong to *Saccharomyces cerevisiae* species.[54] However, the *S. boulardii* strains that are used therapeutically to treat human gastrointestinal tract disorders show tight clustering, both genetically and metabolically.[55]

S. boulardii is absent from the natural gut microbiota. If administered, it achieves steady-state concentrations in the colon within 3 days and is cleared from the stools 2–5 days after discontinuation.[56,57] In mice, *S. boulardii* reached 10^7 colony forming units (CFU)/g of feces in a steady state, when 5×10^8 CFU was administered daily. When the administration was stopped, the yeast still numbered 7.3×10^6 CFU/g 3 days later, but was undetectable after 1 week.[58]

Compared with bacterial probiotics, the yeast cells of *S. boulardii* have the following advantages: they are antibiotic resistant due to their fungal nature, and they do not exchange DNA, eg, resistance genes with bacteria.[53]

Although the use of *S. cerevisiae* (eg, *S. boulardii* CNCM I-745) is considered safe, an increased number of *S. cerevisiae* infections (fungemia) have been observed in critically ill and/or immunocompromised patients.[59] Interestingly, virulence seems to be associated with an enhanced tolerance to oxidative stress[60] as well as increased copy numbers of genes of the purine nucleotide synthesis pathway, which in turn increase survival rates in the bloodstream of the host.[61] However, in order to cause fungemia, *S. cerevisiae* first needs to cross the intestinal barrier and reach the bloodstream in sufficient numbers.

S. boulardii has all necessary prerequisites as probiotic. Other than its close relative *S. cerevisiae*, it has a growth temperature optimum around 37°C and a relatively high acid tolerance.[62,63]

A substantial part of the efficacy of *S. boulardii* is dependent on its vitality,[64,65] which can differ with preparation and storage techniques, lyophilized (freeze-dried) *S. boulardii* CNCM I-745 being clearly superior to heat-dried preparations regarding revitalization speed and growth.[66]

Accordingly, for the selection of therapeutic or preventive yeast products, attention should be paid not only to the used strain but also to its preparation.

Clinical efficacy of *S. boulardii*

Numerous clinical studies, almost all performed with lyophilized *S. boulardii* CNCM I-745, demonstrate efficacy and safety for a variety of gastrointestinal conditions associated with diarrhea.[67–69] In contrast to other probiotics, *S. boulardii* was found to have a very broad clinical efficacy with significant positive effects in many different dysbiotic situations.[70]

For example, there is excellent evidence from 14 of 17 total studies that *S. boulardii* can prevent antibiotic-associated diarrhea.[71] Also, *S. boulardii* can be of advantage in preventing or reducing *C. difficile*-associated colitis[72,73] or traveler's diarrhea.[74] Furthermore, the administration of *S. boulardii* can significantly shorten infectious diarrhea in children,[75,76] and the incidence of tube-fed-associated diarrhea in enterally fed patients decreases with *S. boulardii* administration.[77–80]

S. boulardii – mechanisms of action

There are several different effects induced by *S. boulardii*, both during a dysbiotic situation and in the prevention of dysbiosis (Table 1).[69] Most of these effects are also cataloged in a

recent review.[81] Various mechanisms of action, outlined in what follows, have an influence on the microbial population (effects on the microbiota to be described in "The effect of S. *boulardii* on the microbiota") by directly or indirectly improving the growth environment for beneficial microbiota.

General immune stimulation

It is well known that yeast cells are an excellent source of $\beta(1,3)$D-glucan. β-Glucans are fungal wall components that act as "biological response modifiers" due to their ability to activate the immune system.[82,83] Glucans bind to specific receptors on dendritic cells (dectin-1) as well as to receptors on innate immune cells (eg, Toll-like receptors, complement receptor-3).[84,85]

A general immune stimulation is of advantage to the host, enabling the destruction of pathogens at an early stage. This yeast-mediated effect is particularly important as a preventive measure in a healthy situation – keeping the microbial environment in status quo – ensuring the bioreactor runs smoothly.

Anti-inflammatory and antisecretory action

The level of inflammation during diarrheic dysbiosis varies, depending also on the cause of the dysbiosis.

In patients with chronic idiopathic diarrhea, no leukocytes were detected within the fecal mucus layer, indicating the absence of a local cellular inflammatory response.[18] Yet there are many situations when diarrhea is associated with inflammation. The inflammatory response is elicited by bacteria, which manage a direct contact to the mucosa, due to a destabilized mucus layer, and infiltrate the tissue. In response, an increased number of leukocytes migrate into the intestinal lumen to hinder bacterial infection.

Inflammatory effects, along with the symptomatic consequences of diarrhea, and cramping, do have their role in reducing infection, but the attempts to get rid of possibly lethal invaders also open the door to new infections, eg, by making the mucus layer less viscous and thus more permeable for microbes.

Many different studies demonstrate that *S. boulardii* elicits, also via secreted factors, pronounced anti-inflammatory and antisecretory effects by affecting key signaling pathways in intestinal host cells, such as the NF-κB and the MAP kinase pathways, which regulate the tight junction barrier as well as inflammation.[86–90] The antisecretory properties of *S. boulardii* regarding water and electrolyte secretion were also demonstrated in additional studies.[91–94]

Reducing inflammation and secretion has a clear symptomatic advantage, and goes along with a faster recovery of the microbiota by offering an intestinal environment more like that during healthy conditions.

Prebiotic effect

The cell wall material of *S. boulardii* is composed of glucans, mannoproteins, and chitin, which serve as excellent substrates for microbial fermentation, especially for various SCFA producers. This helps explain the increase in butyrate and other SCFA produced within the colon after *S. boulardii* administration.[95,96]

In a long-term in vitro rumen simulation, both living and autoclaved *S. boulardii* significantly stimulated SCFA production, with no major differences between the treatments,[97] suggesting a prebiotic effect of the yeast. Further studies are necessary to evaluate the extent of this effect in a clinical situation.

Trophic effect on enterocytes

Moreover, *S. boulardii* is able to synthesize and secrete polyamines. These polyamines can be absorbed by enterocytes and have a positive effect on their maturation as they play an important role in cell proliferation and differentiation. Furthermore, polyamines enhance the expression of intestinal enzymes.[98–100]

Table 1 Mechanisms of action of *Saccharomyces boulardii* CNCM I-745

Probiotic: creation of a favorable microbiotic environment	Mucus protection; elimination of bacterial toxins, pathogen binding	Possible prebiotic (substrate for beneficial microbiota) effect and stimulation of digestive enzymes	General immune stimulation; anti-inflammatory and antisecretory action
In case of dysbiosis	Protection against pathogens and their toxins; regeneration of mucosal cells	Regeneration and stabilization of healthy microbiota; improved enzymatic function of mucosal cells	Reduction of secretory water and electrolyte loss; reduction of inflammation
Preventive action	Protection against pathogens and their toxins	Stabilization of healthy microbiota; improved enzymatic function of mucosal cells	General healthy stimulation of the immune system

Elimination of bacterial toxins, pathogen binding, and growth inhibition of microbial pathogens

S. boulardii produces factors that neutralize bacterial toxins and modulate host cell signaling pathways implicated in proinflammatory response during bacterial infection.[53] For example, *S. boulardii* releases a protease that cleaves *C. difficile* toxins;[101] also, *S. boulardii* can inactivate cholera toxin[102] and dephosphorylate lipopolysaccharides from *Escherichia coli* O55B5.[103]

In addition, a direct binding to some pathogens is possible: strains of *E. coli*, *Salmonella typhimurium*, and *S. typhi* adhere to the surface of *S. boulardii*, thus preventing adhesion to and invasion of the host.[104–106] Moreover, *S. boulardii* can inhibit the growth of a number of microbial pathogens like *S. typhimurium*[107] and *C. albicans*.[108]

Physical barrier effect and colonization resistance

The presence of an intact mucus layer is pivotal to the protection of the intestinal mucosa against bacterial infiltration.[15]

It can be hypothesized that the sticky outer mucus surface offers the opportunity for *S. boulardii* to grow and build protective interlaced layers, making it even more difficult for pathogenic strains to reach the mucosa.[19]

In the healthy situation, commensals have been shown to limit pathogen colonization by competing for metabolites, thereby leading to "colonization resistance."[109] Similarly, *S. boulardii* – by its mere presence and/or its metabolic activity – may hinder potentially harmful bacteria from occupying a niche at the exposed mucosa. At the same time, *S. boulardii* itself is not competitive enough to keep this position for long, once habitual/essential bacteria regenerate. Yet its makeshift function may give the host further opportunities to rebuild the mucus layer by reducing the pressure from the potentially harmful microbiota. Studies are needed to verify this hypothesis.

The effect of *S. boulardii* on the microbiota
Overview

Through its multiple mechanisms of actions, *S. boulardii* causes a faster reestablishment of a healthy microbiome following dysbiosis. This has been demonstrated in several human studies as well as in several animal models (Tables 2 and 3).

The effects of *S. boulardii* (most, if not all, studies on strain CNCM I-745) were studied mainly by analysis of the feces – this gives a rather accurate picture of the lower colon; however, the microbial situations in the less densely populated upper intestinal sections have not been studied in relation to the influence of *S. boulardii*.

Feces are inhomogeneous by nature, with different bacteria localized within the core than within the fecal mucus. Unfortunately, not all studies have taken this fact into account, and this may have led to slightly differing results.

Nonclinical studies

To examine the effects of *S. boulardii* in antibiotic-induced diarrhea, two studies were performed in a human microbiota-associated mouse model,[58,137] and one study used a Syrian hamster model.[136] All three studies examined the microbiota before, during, and after antibiotic treatment. No antibiotic controls were applied to study the effect of *S. boulardii* in the healthy situation.

The common results were that treatment of *S. boulardii* did not have any significant effects on the healthy microbial composition. However, following antibiotic treatment, *S. boulardii* caused a significantly more rapid recovery of the normal intestinal microbiota.

Additionally, the effects of *S. boulardii* in obese, type 2 diabetic db/db mice were studied.[111] For this, a prior publication also needs to be considered, which describes the altered microbiota of obese, type 2 diabetic db/db mice compared with normal mice.[110] Altogether, it can be derived that the treatment of *S. boulardii* renders the microbiota of the obese, type 2 diabetic db/db mice more "normal," corresponding to the observed reduction of the low-grade inflammation and fat mass (Table 2).[111]

Clinical studies

The overview of clinical studies is explained in Table 3.

Effects of *S. boulardii* on the healthy human microbiome

Two studies observed the effects of *S. boulardii* on the fecal microbial composition of healthy children/healthy volunteers. Whereas one study[112] found a massive reduction of culturable *E. coli* within feces of children treated with *S. boulardii*, another study[113] did not find any differences in the predominant fecal microbiota with PCR (polymerase chain reaction) analysis. Also culture-based techniques did not find significant changes in the microbiota in healthy controls receiving *S. boulardii*.[114]

DNA isolation from feces, followed by 16S rRNA gene PCR amplification and denaturing gradient gel electrophoresis for fingerprinting did not detect any universal changes in the fingerprints of volunteers treated with *S. boulardii*.[115]

Table 2 Nonclinical studies regarding the influence of the administration of *Saccharomyces boulardii* CNCM I-745 on the composition of the intestinal microbiota

Study	Model	Effects of *S. boulardii* on microbiota (and some other results)

Philippe-Taine[136]	Syrian hamster model
	• 4×10^{10} CFU *S. boulardii*/kg/d; 5 hamsters; day 1–12
	• control hamsters; day 1–12
	  in both groups: 1× clindamycin, 1 hour after aforementioned treatment on day 1
	  Feces analysis by aerobic bacterial culture until day 18
		No effects of *S. boulardii* on healthy aerobic flora
		Effects by *S. boulardii* on aerobic flora:
		• decrease in the amplitude of antibiotic-induced changes
		• reduction of resistant streptococci, Enterobacteriaceae, Candida
		• more rapid recovery of basal level of staphylococci

Barc et al[58]	Human microbiota-associated mouse model
	A 7-day (day 1–7) oral treatment with amoxicillin–clavulanic acid:
	• Day 1–14: *S. boulardii* (5×10^8 CFU/d), 6 mice
	• Day 1–14: water (control), 6 mice
	  Feces analysis before, during, and after antibiotic treatment up to day 29
	  FISH with group-specific 16S rRNA probes + flow cytometry
		Antibiotic treatment: increase of Enterobacteriaceae and *Bacteroides*; dramatic decrease of *Clostridium coccoides–Eubacterium rectale* (Lachnospiraceae)
		S. boulardii: Significantly more rapid recovery of normal intestinal microbiota in the *S. boulardii*-treated mice compared with the control mice ($P=0.05$)

Collignon et al[137]	Human microbiota-associated mouse model
	A 7-day (day 1–7) oral treatment with amoxicillin–clavulanic acid:
	• Day 1–14: *S. boulardii* (5×10^8 CFU/d), 6 mice
	• Day 1–14: control, 6 mice
	  Feces analysis before, during, and after antibiotic treatment up to day 29
	  FISH with group-specific 16S rRNA probes: mainly flow cytometry
		Antibiotic treatment: disrupted intestinal microbiota: *C. coccoides* group (Lachnospiraceae) decreased; *Bacteroides* group increased; Enterobacteriaceae became detectable
		S. boulardii: more rapid restoration of the balance of the dominant anaerobic microbiota
		Other *S. boulardii* effects: downregulation of antigen-presenting function of dendritic cells (anti-inflammatory effect)

Everard et al;[111] Geurts et al[110]	Obese, type 2 diabetic db/db mice: • 4 weeks *S. boulardii*, 15 mice • 4 weeks saline (control), 15 mice DNA isolation, 16S rRNA gene PCR amplification followed by sequencing	db/db mice: significant decrease in *Bacteroidetes*, significant increase of *Firmicutes* and *Proteobacteria* compared with lean mice *S. boulardii*: increase in Bacteroidetes (increase in Bacteroidaceae, decrease in Porphyromonadaceae) and decrease in Firmicutes (including *Ruminococcus*), Proteobacteria, and Prevotella. Other *S. boulardii* effects: reduction of hepatic steatosis, low-grade inflammation, and fat mass, increase of cecum weight and cecum tissue weight

Abbreviations: CFU, colony forming units; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.

These findings can be confirmed using fluorescence in situ hybridization (FISH) probing – the structural organization of fecal microbiota in healthy subjects was stable and unaffected by *S. boulardii*.[18,49]

Also, 16S rRNA gene pyrosequencing did not reveal any substantial modification by *S. boulardii*.[116]

Accordingly, and in correspondence with the nonclinical results, *S. boulardii* does not seem to alter the composition of the healthy microbiota, except perhaps for a certain reduction of the rather minor prevalence of Enterobacteriaceae like *E. coli*.[112]

This appears reasonable. In a healthy situation, *S. boulardii* is certainly not competitive enough to displace the healthy microbiota, and the number of *S. boulardii* during healthy intestinal passage may possibly not rise above the administered dose.

On their way through the healthy intestinal tract, most yeast cells become digested. A preliminary unpublished investigation on ten healthy students pointed toward slightly reduced total bacterial fecal numbers, when these students were administered *S. boulardii*, possibly indicating slightly more efficient overall food consumption, with reduced chances of bacterial growth at the stage of excretion (data not shown).

Altogether, the dysbiosis prevention (Table 1) is plausibly derived from the aforementioned ("*S. boulardii* – mechanisms of action") mechanisms of action of *S. boulardii*.

Both the yeast cell wall components stemming from the digested yeast cells as well as those yeast cells that managed to pass the acid stress of the stomach alive and begin to prosper within the intestine, unfold multiple preventive actions.

Table 3 Overview of reviewed clinical studies regarding the influence of the administration of *Saccharomyces boulardii* (CNCM I-745 unless indicated) on the composition of the intestinal microbiota

Clinical study	Study population and design	Effects of *S. boulardii* on microbiota (and some other results)	Remarks

Girard-Pipau et al,[114] Schneider et al[95]	25 participants receiving 500 mg *S. boulardii* tid orally/via tube for 6 days (days 0–5) *Two groups:*
	• 10 patients on total enteral nutrition (20% protein, 45% carbohydrate, and 31% fat) without diarrhea:
	  ○ 7 men, 3 women
	  ○ median age 59.2±5.5 years
	• 15 control patients (regular Western diet):
	  ○ 11 men, 4 women
	  ○ median age 31.9±2.0 years
	Fecal analysis by culture-based methods: 1+2 days before; day: 4+5; day: 14+15
		Before *S. boulardii* treatment:
		Peptostreptococci and *Bifidobacterium* only present in controls
		Compared with controls: more enterococci in patients, more aerobes/less anaerobes in patients
		S. boulardii treatment effects – patients:
		• Some clostridia and some other bacteria dropping
		• *Bifidobacteria* appearing
		• Normalization of bacterial SCFA in patients to control levels: from 107.5±18.2 to 150.2±27.2 mmol/kg, *P*=0.02
		• Total SCFAs that remained high 9 days after treatment was discontinued
		S. boulardii treatment effects – controls:
		• Decrease of Gram-positive anaerobes from 8.6±0.3 to 6.8±0.8 log CFU/g (*P*=0.035) SCFA: from 113.0±15.2 to 129.0±28.6 mmol/kg, not significant
			Controls are not entirely comparable due to age difference
			Culture-based evaluation of microbiota
			Patients had no diarrhea

Vanhoutte et al[115]	30 healthy subjects, crossover study 3 groups (10 subjects each), including other control periods and: • 4 weeks, 250 mg (2.5×10⁹ CFU)/d *S. boulardii* • 4 weeks, 500 mg (5×10⁹ CFU)/d *S. boulardii* • 4 weeks, 250 mg/d *S. boulardii* + lactulose DNA isolation, 16S rRNA gene PCR amplification, followed by DGGE for fingerprinting	*S. boulardii*: No detectable universal changes in DGGE profiles Increase (*P*<0.05) in total Bifidobacteriaceae with lactulose (not with *S. boulardii*)	Healthy
Akil et al[112] "Commercial capsules"	24 children, 3–16 years (mean 8.7±3.8), 14 boys and 10 girls receiving 5×10⁹ CFU *S. boulardii* once/day for 5 days Immediate fecal analysis regarding *Escherichia coli* and yeast via culture technique: before + day 5	*S. boulardii*: Mean number of *E. coli* (CFU): from 384,625±445,744/g stool to 6,283±20,283/g stool (significant) Mean number of *S. boulardii* (CFU): from 0 to 11,047±26,754/g stool	*E. coli* and *S. boulardii* evaluated Healthy children
De Preter et al[113]	43 healthy volunteers Influence of lactulose and/or *S. boulardii*: single dose or after 4-week intake Urine and feces analysis PCR: predominant fecal microbiota	No significant effects were observed after *S. boulardii* intake	Healthy

Swidsinski et al,[49] Swidsinski et al[18]	40 participants: 3 weeks observation, 3 weeks oral *S. boulardii* 250 mg tid, 3 weeks follow-up
	• 20 patients with chronic idiopathic diarrhea
	  ○ 11 men and 9 women
	  ○ 25–72 years (mean 48.2 years);
	• 20 healthy controls
	  ○ 8 men and 12 women
	  ○ 18–60 years (mean 41.2 years);
	Feces analysis every week for 9 weeks, 100% compliance
	FISH probing of immediately fixed stool cylinders
		Dysbiotic feces in diarrhea (versus normal):
		• Increased mucus depositions within feces
		• Increase of mucus septa and striae
		• Marked reduction of habitual *Eubacterium rectale* (Lachnospiraceae), *Bacteroides*, and *Faecalibacterium prausnitzii* (Ruminococcaceae)
		• Increased concentrations of occasional bacteria
		• Suppression of bacterial fluorescence in fecal center
		• Increased concentrations and spatial shift of mucotrop bacteria to the fecal core
		S. boulardii treatment effects in patients:
		• Partial (40%) or complete (30%) normalization of diarrheal symptoms.
		• Increase in habitual *E. rectale* and *Bacteroides* groups (still below healthy level)
			Work addresses spatial location of fecal microbiota, sampling procedure allows unaltered analysis
			Significant effects of *S. boulardii*

Reference	Methods	Findings	
Osowska et al[117]	• 14 SBS adult patients on long-term parenteral nutrition • 10 healthy untreated control persons • 250 mg *S. boulardii* tid for 2 weeks FISH probing of immediately fixed stool cylinders at days 0, 7, 14	• Decline in the substitution of habitual with "occasional" bacterial groups • Decrease of mucotrop bacteria together with reduction of mucus and a firmer stool structure No treatment effects in healthy subjects: Unaffected structural organization of fecal microbiota SBS specific increase in Lactobacilli, alterations of normal microbiota *S. boulardii* in SBS: increase in Bacteroidaceae/Prevotellaceae (Bac303) and *Roseburia* (Lachnospiraceae); decrease of Bifidobacteriaceae and Enterobacteriaceae. These are (not complete) changes toward normal levels. Also, the concentration of plasma lactate fell to reference values: from 30.72 mg/dL to 18.64 mg/dL	Conference abstract
Kelly[116] "Dietary Supplement"	48 healthy volunteers, 18–65 years Open label, randomized pilot study 4 groups, 12 each: • *S. boulardii* 500 mg, 2 times daily for 14 days • Amoxicillin/Clavulanate 875 mg/125 mg 2 times daily >1 h before meals for days 1–7 • Same antibiotics, days 1–7; in addition *S. boulardii*, days 1–14; 500 mg, tid • Control: no intervention 7 stool samples/group; GI symptom questionnaires; 16S rRNA gene pyrosequencing for bacterial genera detection	Prior to antibiotic treatment: individual enterotypes Antibiotic: Reduced prevalence of *Ralstonia*; increases in *Parabacteroides* and *Escherichia/Shigella* significantly correlated with diarrhea. Antibiotic + *S. boulardii*: Changes significantly attenuated *S. boulardii* alone: no substantial microbiome modification, prevention of diarrhea	

Swidsinski,[118] Swidsinski et al[119]	60 women (initially 30) treated for bacterial vaginosis, receiving metronidazole (3×400 mg/d) and ciprofloxacin (2×500 mg/d)
	Group I: antibiotics for weeks 1 and 2
	Group II: 250 mg *S. boulardii* tid concomitant to antibiotics for weeks 1 and 2
	Group III: antibiotics in weeks 1 and 2 followed by 250 mg *S. boulardii* tid in weeks 3 and 4
	FISH analysis of Carnoy fixated stool cylinders from days –90, –60, –30, 7, 14, 28, 42, 56, and 70
		Prior to antibiotic treatment:
		• Individual stable enterotypes
		  ○ Habitual/essential bacteria Bacteroidaceae, *F. prausnitzii* (Ruminococcaceae) and *Roseburia* (Lachnospiraceae)
		  ○ Other substantial bacteria from various genera, differing interindividually
		Antibiotic:
		• Suppression of habitual/essential bacterial groups followed by slow recovery
		• Emergence of accidental bacteria (previously very minor) during antibiotic treatment, which, however, soon become out-competed by the pioneers
		• Emergence of pioneer bacteria, peaking 2–4 weeks after end of treatment: Bifidobacteriaceae, Enterobacteriaceae or *Clostridium lituseburense* (*difficile*) (Peptostreptococcaceae); reduction of pioneer bacteria back to original levels upon restoration of substantial bacteria
		• Increase of volatile microbial groups, change in microbial profile
		Antibiotic + *S. boulardii*: Reduction of antibiotic-associated suppression of bacteria
		Antibiotic followed by *S. boulardii*: Faster recovery of original microbiota, within 4 weeks after end of treatment
		S. boulardii: reduction of volatility, significantly fewer changes in microbial profile	

Abbreviations: tid, three times a day; SCFA, short chain fatty acid; CFU, colony forming unit; PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; SBS, short bowel syndrome; FISH, fluorescence in situ hybridization; GI, gastrointestinal.

Owing to the already well-functioning intestinal microbiome in a healthy human, the preventive effects of *S. boulardii* become apparent only during special challenges, eg, during exposure to pathogens eliciting traveler's diarrhea,[74] or in the prevention of antibiotic-induced diarrhea.[71] However, these effects are indicative that – without having any pronounced influence on the composition of the healthy microbiome – *S. boulardii* is capable of protecting this microbial community.

Effects of *S. boulardii* during dysbiosis

In a culture-based evaluation of the effects of *S. boulardii* in patients on (low-fiber) total enteral nutrition, the yeast improved the overall microbial fecal composition – even in patients who were not suffering from diarrhea.[95,114] Before treatment, many more different bacterial strains could be isolated from the feces of the healthy controls as compared with the feces of patients, indicating a somewhat reduced diversity in the patients. *S. boulardii* led to a number of changes of the microbiota, eg, reducing counts of certain bacteria, some of which recovered after stopping the treatment, while others did not. This included the reduction of several clostridial strains. Also, there seemed to be an overall reduction of Gram-positive anaerobes in the controls.

In view of the culture-based evaluation of the microbiota, the results must be interpreted with caution. It is possibly more interesting that the authors noted a significant increase in SCFA after 6 days of *S. boulardii* administration. In particular, butyrate and propionate increased significantly within the feces of the tube-fed patients. In the healthy controls too, there was a slight increase in SCFA, but it was nonsignificant.

With the current understanding, it can be assumed that the increase in SCFA in the patient group is an indirect consequence of the *S. boulardii*-induced return of the gut microbiota to a more normal composition.

A major contribution has been the study of patients with chronic idiopathic diarrhea as compared with healthy controls.[18,49] This study used an advanced method of detection – FISH probing of immediately fixed stool cylinder specimens along with a spatial analysis of the microbiota, allowing a differentiation between mucus layer and central fecal microbiota. Accordingly, the fecal ecosystem is accurately described both in the healthy situation and during diarrheic dysbiosis. The latter caused a drastic increase of
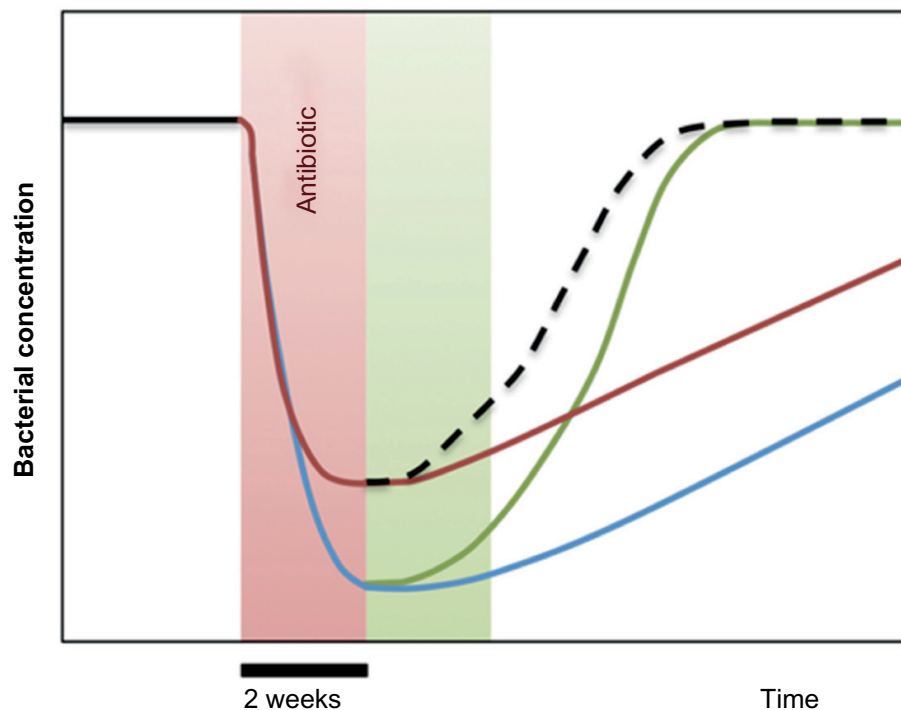

Figure 2 Generalized scheme of the effects of antibiotic dysbiosis on habitual/essential bacteria and other substantial bacteria.
Notes: Upon a 2-week antibiotic treatment (red area), the main microbial population suffers an abrupt decrease (blue line). *Saccharomyces boulardii* administered during antibiotic treatment (red area; red line) can reduce this decline by protecting the microbiota. If instead *S. boulardii* is administered following antibiotic treatment (green area; green line), *S. boulardii* can support a faster regeneration of the microbial population. Accordingly, the optimum would be a combination of both, a treatment with *S. boulardii* during and following antibiotic treatment. This is represented by the hypothetical black dotted line, which was derived from the other lines. The worst-case scenario is no *S. boulardii* treatment (blue line). Pioneer bacteria and accidental bacteria are excluded.

the mucus layer (assumable along with a lower viscosity and thus higher permeability) and profound changes in the composition of the microbiota. Almost all parameters typical of diarrhea improved significantly with *S. boulardii* treatment, and most changes persisted after cessation of therapy. This went along with a partial (40%) or complete (30%) normalization of diarrheal symptoms.

In a study with 14 Short Bowel Syndrome (SBS) adult patients on long-term parenteral nutrition, *S. boulardii* also partially rendered the microbiota more similar to the healthy controls. Also here, FISH probing of immediately fixed stool cylinder specimens was used as methodology.[117]

A study with 60 women (30 in preanalysis, 60 in soon to be published final analysis) treated for bacterial vaginosis examined the effects of antibiotic therapy alone, or contemporaneously with *S. boulardii* treatment, or followed by *S. boulardii* treatment.[118,119] Again, FISH analysis of fixated stool cylinders was used to examine the microbiota. Before treatment, stable enterotypes were observed; however, the antibiotics caused a suppression of the habitual/essential (most common) bacteria as well as other substantial bacterial groups. *S. boulardii* could reduce the antibiotic-associated suppression of these bacteria if administered simultaneously. If administered following antibiotic treatment, *S. boulardii* led to a significantly faster recovery of original microbiota. Additionally, *S. boulardii* led to less pre–postmismatches of the microbial population. In the absence of *S. boulardii*, the antibiotic treatment caused significantly more population differences (compared with before treatment) than if *S. boulardii* had been administered.

A generalized scheme of the effects of *S. boulardii* is depicted in Figure 2. From the curves we conclude that the optimal treatment would be to administer *S. boulardii* simultaneously to the antibiotic treatment, plus subsequently for at least 2 weeks.

In a different study, antibiotic-induced dysbiosis and its treatment with *S. boulardii* were studied in healthy volunteers, using 16S rRNA gene pyrosequencing for bacterial genera detection.[116] Prior to treatment, individual enterotypes could be identified. The antibiotic induced significant alterations of the microbial composition, which were significantly attenuated by *S. boulardii*. In a setting where *S. boulardii* was administered in parallel to the antibiotic, the associated diarrhea could be prevented ($P<0.05$).

Altogether, several small but well-designed studies have demonstrated that *S. boulardii* can lead to a faster recovery from diarrheic dysbiosis with regard to the microbial population, as well as to a certain protection of dysbiosis if administered preventively during antibiotic therapy.

Specific effects of *S. boulardii* on mucus and microbiota

Table 4 summarizes the proposed effects of *S. boulardii* on specific microbial subgroups. The following subheadings match the main groups of Table 4.

The healthy fecal microbiota can be divided into habitual/essential bacterial groups, usually present in all healthy humans and other individual substantial bacteria. Other occasional bacterial are present only in minor proportions.

Depending on the individual enterotype, the amount of specific bacteria within the feces can vary (healthy human prevalence in % is presented in Table 4). During dysbiosis, the proportion of different microbial strains changes, and so does the thickness and consistency of the mucus. In the following, the specific effects of *S. boulardii* on the mucus and the major bacterial groups during dysbiosis and recovery are summarized.

Mucus

It is known that certain bacteria, especially certain pathogens, feed on mucin oligosaccharides.[120] This way they are capable of reducing the mucus integrity, leading to increased porosity and maceration, thereby opening a path for themselves and other bacteria to reach the epithelial cells.
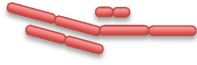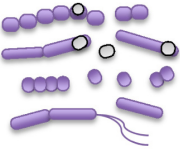
Thus, a polymicrobial infection, sustained by a broken mucus barrier, is characteristic for inflammatory conditions like ulcerative colitis.[121]

A study on chronic idiopathic diarrhea has shown an increase in fecal mucus thickness with more watery stools.[18,49] However, as the fecal mucus layer contained many septae and was often disrupted, the increased thickness ($265\pm266\,\mu m$) of the mucus did not lead to enhanced stability. Upon *S. boulardii* treatment, the mucus layer got reduced to $96\pm118\,\mu m$ ($P=0.002$). After *S. boulardii* treatment was stopped, a reincrease of the mucus layer was observed.

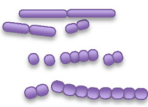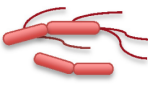### Bacteroidetes – Bacteroidia – Bacteroidales

Bacteroidales are Gram-negative obligate anaerobic rods, playing a fundamental role in the processing of complex molecules to simpler ones within the intestinal tract.[122] Bacteroidaceae (genus: *Bacteroides*) and Prevotellaceae (genus: *Prevotella*) have a prominent prevalence within the healthy colon and can be assigned to the habitual/essential microbiota. The Porphyromonadaceae (including *Porphyromonas* and *Parabacteroides*) are mostly of lower prevalence (Table 4).

Table 4 Bacterial groups in the feces and their major changes in prevalence due to (mostly antibiotic induced) dysbiosis as well as *Saccharomyces boulardii* (mostly strain CNCM I-745) treatment

Intestinal bacterial group with important fecal prevalence Exemplary on genus level	Examples of shape Commonly used probes[a],[141] "Alternate group names"	Gram stain; Aerobic/anaerobic Fermentation products	Healthy human fecal prevalence in %[b]	Significant population changes in studies with *S. boulardii* (Sb)
Phylum: Bacteroidetes Class: Bacteroidia Order: Bacteroidales		Gram-negative nonmotile rods, obligate anaerobes	41.0–47.7[142] 59.2[51] 36.8±16[143]	
Family: Bacteroidaceae • *Bacteroides*	Bfra602-Bdis656 Bac303[c]	Acetate, succinate	22.7[51] 11±7.8[144] (Bfra) 8.5±7.1[134] (Bac303) 20[145] (Bfra602–Bdis656)	Less reduction during antibiotic treatment with Sb[118],[119] ↑ Improved recovery with Sb[18],[117–119]
Prevotellaceae • *Prevotella*	Bac303[c]		31.7[51] 4.4±4.9[144]	
Porphyromonadaceae • *Porphyromonas* • *Parabacteroides*			3.6[51]	↓ Reduction back to normal levels with Sb[116]
Firmicutes Clostridia Clostridiales		Gram-positive rods or cocci, obligate anaerobes	Firmicutes: 49.4–54.8[142] 34.7[51]	
Lachnospiraceae • *Blautia* • *Coprococcus* • *Lachnoclostridium* • *Roseburia*	Erec482 "*Clostridium coccoides– Eubacterium rectale* group" Clostridium cluster XIVa and XIVb "*Roseburia* group"	SCFA, acetate, formate, ethanol, H_2, lactate, succinate	7.5[51] 29±12[144] 7–28[143] 28.0±11.3[134] 29[145]	Less reduction during antibiotic treatment with Sb[118],[119] ↑ Improved recovery with Sb[18,58,117–119,137]
Ruminococcaceae • *Faecalibacterium prausnitzii* • *Ruminococcus bromii* • *Ruminococcus callidus* • *Ruminiclostridium* [*Clostridium*] *leptum*	Fprau Clep 866 (Rbro730+ Rfla729+ Rcal733+ Fprau645) Cvir 1414, Edes 635 S-G-Clept-1240 "*C. leptum* group; Clostridium cluster IV"	Butyrate, acetate, lactate, formate, H_2	6–39[143] 26.7(±7)[134] 15±10[144](S-G-Clept-1240) 6.3[51] 15.4±7.2[134] (Fprau645)	Less reduction during antibiotic treatment with Sb[118],[119] ↑ Improved recovery with Sb[118],[119]
Peptostreptococcaceae • *Romboutsia* [*Clostridium*] *lituseburensis* • *Peptoclostridium* [*Clostridium*] *difficile*	Clit135 "*C. lituseburense* group"	Butyrate, acetate		Increase during diarrhea[18] Pioneer species[118],[119]
Clostridiales Family XI. Incertae Sedis • *Anaerococcus* • *Finegoldia* • *Peptoniphilus*			14.8[51]	
Clostridiaceae • *Clostridium histolyticum*	Chis150 "Clostridium cluster I and II"			↓ Reduction back to normal levels with Sb[118],[119]
Eubacteriaceae • *Eubacterium cylindroides*	Ecyl387	Butyrate	1.1±1.9[134]	Increase during diarrhea[18]
Firmicutes Negativicutes Selenomonadales		Gram-negative		

(Continued)

Table 4 (*Continued*)

Intestinal bacterial group with important fecal prevalence Exemplary on genus level	Examples of shape Commonly used probes[a,141] "Alternate group names"	Gram stain; Aerobic/anaerobic Fermentation products	Healthy human fecal prevalence in %[b]	Significant population changes in studies with *S. boulardii* (Sb)
Veillonellaceae • *Dialister* • *Megasphaera* • *Veillonella*	Veil223	Fermenting lactate → proprionate, acetate	3.5[51] 15±10[144] 1.3±1.3[134]	
Firmicutes **Bacilli** Lactobacillales		Gram-positive rods or cocci, aerotolerant or facultative anaerobes		
Lactobacillaceae • *Lactobacillus* **Enterococcaceae** • *Enterococcus* **Streptococcaceae** • *Streptococcus*	Lab158[c] Lab158[c] Strc493	Lactic acid, ethanol, acetate	1.8±1.4[134] (Lab158) 0.6±0.8[134]	Increase in SBS patients[117] Streptococcus: Prevention of antibiotic-associated peak by Sb[118,119]
Actinobacteria **Actinobacteria**		Gram-positive rods, anaerobes	1.94[51]	
Bifidobacteriales **Bifidobacteriaceae** • *Bifidobacterium*	Bif164	Sometimes aerotolerant Organic acids, SCFA	1.07[51] 0–5[143] 1.2–6[146] 6.0±6.4[144] 4.4±4.3[134]	↓ Reduction back to normal levels with Sb;[117] Pioneer species[118,119]
Coreobacteriales **Coriobacteriaceae** • *Coriobacterium* • *Consinella* • *Atopobium* • *Eggerthella*	Ato291	Obligate anaerobes Acetate, lactate ethanol, H_2	<1 4.9±4.2[144] 1.7±2.3 3.1±2.8[134] 0, 1–7[146]	
Proteobacteria **Gammaproteo-bacteria** Enterobacteriales		Gram-negative rods, facultative anaerobes	2.39[51]	
Enterobacteriaceae • *Escherichia* • *Shigella* • *Enterobacter* • *Salmonella* • *Yersinia*	Ebac1790 Enter1432[134]	Lactate, etc, Nitrate → nitrite	0.1±0.1[134]	↓ Reduction back to normal levels with Sb;[58,116–119,136,137] Diarrheic increase especially in mucus;[18] Pioneer species[118,119]
Alcaligenaceae • *Alcaligenes faecalis* **Campylobacteraceae** • *Campylobacter*	Alc-476	Nitrate → nitrite, denitrifying	1.14[51] 0.92[51]	
Verrucomicrobia **Verrucomicrobiae** Verrucomicrobiales		Gram-negative rods, obligate anaerobes		
Verrucomicrobiaceae • *Akkermansia*	Muc1437	Capsule-forming on mucin, fermenting carbohydrates	Prominent prevalence in mucus	Improved modulation toward normal levels with Sb[18,118,119]

Notes: [a]Accurate specificities on a species or genus level can be derived from literature (usually cited within probeBase); [b]some values are rounded; [c]probes for multiple groups (check other groups as well). Detection by different methods: 1) culture techniques,[136] 2) clonal sequencing,[142] 3) pyrosequencing,[51,116] 4) 16S rRNA dot blot hybridization,[143] 5) 16S rRNA PCR,[144] 6) FISH combined with flow cytometry,[58,134,137] 7) FISH and microscopic counting of homogenized samples,[145,146] 8) FISH and microscopic counting of immediately fixed stool cylinders.[18,49,117–119]
Abbreviations: SCFA, short chain fatty acids; SBS, short bowel syndrome; FISH, fluorescence in situ hybridization; PCR, Polymerase chain reaction; rRNA, ribosomal ribonucleic acid; *Sb, S. boulardii.*

During ulcerative colitis, a strong reduction of the Bacteroidaceae/Prevotellaceae was observed.[19]

Interestingly, Bacteroidales are able to grow on mucin, and this can explain their mucosal colonization in IBD.[123]

As found in a clinical study, diarrhea was associated with a massive reduction in the combined Bacteroidaceae and Prevotellaceae (Bac303 probe[124]).[18,49] During treatment with *S. boulardii*, their amount significantly increased, even though a full recovery to healthy levels could not be observed. Also, in patients with SBS Bacteroidaceae/Prevotellaceae decreased, and upon *S. boulardii* treatment, their amount increased significantly toward more normal levels (no complete normalization).[117] Similarly, following antibiotic-induced dysbiosis, Bacteroidaceae/Prevotellaceae strongly declined; this suppression was attenuated by *S. boulardii*.[118,119] In another study on antibiotic-induced diarrhea, an increase of *Parabacteroides* was noted, and this change was attenuated with *S. boulardii*.[116]

Firmicutes – Clostridia – Clostridiales
Within the class of Clostridia, systematic reclassifications have taken place recently. Table 4 includes their most relevant families for the microbiota.

Also, the most prevalent fecal groups are discussed here:

Lachnospiraceae
Members of this family are also described as "*Clostridium coccoides–Eubacterium rectale* group", "Clostridium cluster XIVa and XIVb", or "*Roseburia* group".[125–127]

They are fermentative, often spore-forming obligate anaerobes, and are well known as major SCFA producers,[126] although other fermentation products are possible.

As a major group within the human feces, they constitute between 7% and 29% (Table 4) of the fecal bacteria in a healthy situation.

Ruminococcaceae
Members of this family are also described as "*Clostridium leptum* group," "Clostridium cluster IV," "*Faecalibacterium prausnitzii* group," or "*Ruminococcus* group."[125–127]

Similarly to the Lachnospiraceae, they are fermentative, obligate anaerobes, and major butyrate producers;[126] other fermentation products are acetate, lactate, formate, and hydrogen. In the healthy human, they constitute between 6% and 39% (Table 4) of the fecal bacteria.

A reduction of Ruminococcaceae was described in Crohn's disease and ulcerative colitis patients.[128] Indeed, *F. prausnitzii*, which has a very strong prevalence in the

healthy, is extremely sensitive to intestinal disturbances, and thus, the undetectability of *F. prausnitzii* in stool samples is indicative of active Crohn's disease.[19] Interestingly, anti-inflammatory effects are linked to this bacterium.[129,130]

The prevalence of both Lachnospiraceae and Ruminococcaceae was reduced during idiopathic diarrhea.[18,49] A statistically significant increase, although not to healthy levels, of the Lachnospiraceae could be observed due to *S. boulardii*.[18,49] Similarly, in SBS patients, the Lachnospiraceae were reduced, and an increase could be observed with *S. boulardii* treatment.[117]

The reduction of these clostridial groups during diarrheic dysbiosis goes along with the observed decrease in SCFA; their increase upon treatment with *S. boulardii* is paralleled by an increase in SCFA.

Firmicutes – Bacilli – Lactobacillales
The Lactobacillales, including Lactobacillaceae, Enterococcaceae, and Streptococcaceae, only make up a minor portion of the microbiota within the healthy colon (Table 4). However, these groups can serve as starter cultures not only in food fermentation, but also during regeneration of the microbiota after dysbiosis – this is why members of the Lactobacillales are also widely used as probiotics.[131–133]

In patients with short bowel syndrome, a significant increase in the concentration of Lactobacilli was observed, with a tendency to decrease with the administration of *S. boulardii*.[117]

Actinobacteria – Actinobacteria – Bifidobacteriales – Bifidobacteriaceae
Bifidobacteria can be considered equivalent to pioneer plants after clear-cutting, vastly increasing after dysbiosis, but giving up their makeshift position upon regeneration of the entire microbiome. As such, they are excellent indicator microbes to identify dysbiotic situations, eg, after a stroke.[42]

Several studies have shown an increase in Bifidobacteriaceae during different types of diarrhea.[18,117–119] A return to healthy levels after *S. boulardii* treatment has been demonstrated in SBS patients.[117]

S. boulardii concomitant with antibiotics reduced the antibiotic-associated suppression also of Bifidobacteriaceae, resulting in a slightly stronger interim peak typical of pioneer bacteria.[118,119]

Proteobacteria – Gammaproteobacteria – Enterobacteriales – Enterobacteriaceae
Many intestinal Enterobacteriaceae can be considered mucotrop, with higher numbers within or close to the fecal

mucus layer. However, the central feces also contains (different) Enterobacteriaceae in low abundance, with about 0.1% prevalence within the healthy human feces.[134] Enterobacteriaceae are facultative anaerobes, and can, during anaerobic conditions, as those in the intestine, live on the fermentation of various carbohydrates to lactate and other metabolites. The ability of most Enterobacteriaceae to also gain energy by dissimilatory nitrate reduction gives them a special advantage during inflammation, which makes increased amounts of nitrate available together with an excess supply of undigested carbohydrates.[120]

A special case is the use of the respiratory electron acceptor tetrathionate for anaerobic ethanolamine degradation, which confers a growth advantage on certain *Salmonella* strains. Tetrathionate develops during infection from the oxidation of endogenous sulfur compounds by reactive oxygen species.[135]

Altogether, studies have observed a pioneer-type increase in the number of Enterobacteriaceae during dysbiosis,[118,119] particularly in the mucus layer.[18] Especially problematic is the increase of pathogenic Enterobacteriaceae such as *S. typhimurium* or certain *E. coli* strains.

In the studies with *S. boulardii*, it was found that *S. boulardii* led to a beneficial reduction of Enterobacteriaceae[58,116–119,136,137] – back to healthy levels.

Verrucomicrobia – Verrucomicrobiae – Verrucomicrobiales – Verrucomicrobiaceae
Akkermansia is a bacterium indicative of mucus environment. Following antibiotic treatment, a massive increase in the prevalence of Verrucomicrobia, specifically *Akkermansia muciniphila*, has been observed.[138]

With chronic idiopathic diarrhea, its prevalence increases together with the increase in the thickness of the mucus layer and the number of fecal mucus striae.

However, it should be noted that in other conditions as obesity, the numbers of *Akkermansia* decrease below the normal level, indicative of a thinner, weakened mucus, permitting metabolic endotoxins to enter the bloodstream and cause the chronic inflammation associated with obesity.[139]

It is plausible that the prevalence of *Akkermansia* again gets reduced toward a more normal level upon *S. boulardii* therapy in chronic idiopathic diarrhea, correlated with a reduction of the excessive (but unstable) mucus.[18]

Following antibiotic-induced dysbiosis, *Akkermansia*, and specifically *A. muciniphila*, gets strongly reduced and only regenerates slowly; this regeneration back to normal levels can be significantly supported by *S. boulardii*[118,119] and

is likely to go along with the regeneration of a stable mucus layer of sufficient thickness.

Other members of the microbiota
The literature describes an increasing number of minor genera as potential microbiota inhabitants. At the same time, the nonbacterial microbiota like archaea, fungi, eukaryotes, and viruses also have their place within this complex ecosystem. Especially the role of methanogenic archaea within the colon requires more attention.[140] Altogether, a description of what happens to these other members of the microbiota during different types of dysbiosis and during *S. boulardii* treatment is awaiting future research.

Discussion and conclusion – outlook on the potential of *S. boulardii*
One of the challenges in investigating the composition of the intestinal ecosystem is the detection of minor species within a fecal sample – or finding the needle in a haystack of up to 1,000,000,000,000 bacteria/g. Fortunately, the methodology for a quantitative detection of microbes within a fecal sample has greatly improved in recent years, and will continue to do so. Another challenge is the elucidation of the spatial relationship of different microbial species in health, disease, and therapy.

Previous studies have demonstrated great potential for *S. boulardii* CNCM I-745 in the recovery from diarrhea or its prevention, eg, regarding a reduction of antibiotic-associated suppression of the microbiota as well as their faster regeneration after antibiotic therapy. Investigations on an increased (initial) dosage as well as an elongated treatment scheme, during and also subsequent to antibiotic treatment, could further improve the protection and recovery from antibiotic-induced dysbiosis.

Other dysbiotic conditions too have been shown to profit from *S. boulardii*, eg, SBS, chronic idiopathic diarrhea, or tube feeding-induced diarrhea.

Owing to its multiple mechanisms of actions, *S. boulardii* is promising also for the treatment of dysbiotic situations, which differ from the classical diarrhea, eg, low-level intestinal inflammatory conditions or even constipation. Furthermore, the application of *S. boulardii* could possibly prevent the spread of many more diarrheic outbreaks, also in the developing world, where diarrhea is still a common cause of death, especially in children.

An additional potential of *S. boulardii* lies in a general stabilization of the gut flora for at-risk populations of the

industrialized world, namely, the elderly, people with diabetes, overweight people, smokers, or people with high stress levels (Figure 1; risk factors).

More studies are needed to explore the full potential of the versatile probiotic yeast.

Acknowledgments

Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

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