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Review article: yeast as probiotics – *Saccharomyces boulardii*

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SUMMARY

Background
Probiotics are defined as live micro-organisms which confer a health benefit on the host. Although most probiotics are bacteria, one strain of yeast, *Saccharomyces boulardii*, has been found to be an effective probiotic in double-blind clinical studies.

Aims
To compare the main properties that differentiates yeast from bacteria and to review the properties of *S. boulardii* explaining its potential benefits as a probiotic.

Methods
The PubMed and Medline databases were searched using the keywords 'probiotics', 'yeast', 'antibiotic associated diarrhea', '*Saccharomyces boulardii*', 'bacterial diarrhea' and 'inflammatory bowel disease' in various combinations.

Results
Several clinical studies have been conducted with *S. boulardii* in the treatment and prevention of various forms of diarrhoea. Promising research perspectives have been opened in terms of maintenance treatment of inflammatory bowel diseases. The mechanism of *S. boulardii*'s action has been partially elucidated.

Conclusion
Saccharomyces boulardii is a strain of yeast which has been extensively studied for its probiotic effects. The clinical activity of *S. boulardii* is especially relevant to antibiotic-associated diarrhoea and recurrent *Clostridium difficile* intestinal infections. Experimental studies clearly demonstrate that *S. boulardii* has specific probiotic properties, and recent data has opened the door for new therapeutic uses of this yeast as an 'immunobiotic'.

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INTRODUCTION

The gastrointestinal (GI) microflora ('microbiota') is an extremely complex ecosystem that coexists in equilibrium with the host. When this equilibrium is disrupted, clinical disorders may occur. Microbiota plays a well-established role in infectious GI diseases. Recent research has linked intestinal microbiota disequilibrium to such GI disorders as antibiotic-associated diarrhoea (AAD), ulcers, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and colon cancer. Furthermore, the microbiota has been proposed as a major regulator of the immune system outside the gut. Attempts have been made to improve the health status of affected individuals by modulating the indigenous intestinal flora using living microbial adjuncts called 'probiotics'.

Probiotics have been defined as viable micro-organisms that (when ingested) have a beneficial effect in the prevention and treatment of specific pathological conditions.[1] In fact, probiotics have been used for as long as people have eaten fermented foods. In the early 20th century, the Russian immunologist Elie Metchnikoff suggested that lactobacilli ingested in yogurt could have a positive influence on the normal microbial flora of the intestinal tract.[2] He hypothesized that lactobacilli were important for human health and longevity. In recent years, the definition of a probiotic has changed, primarily because of the recognition that probiotic bacteria can influence the physiological outcomes, distant from the gut lumen. Moreover, the activation of local mucosal protective mechanisms and the modulation of adaptative immune effector functions can influence protection levels and the degree of inflammation at all mucosal sites. These observations shifted the concept of probiotics from a narrow range of dairy isolates that fermented milk and could 'promote health' to the concept of 'immunobiotics'.[3]

Because viable and biologically active micro-organisms are usually required at the target site in the host, it is essential that the probiotic be able to withstand the host's natural barriers against ingested micro-organisms. Most probiotic micro-organisms are bacteria. Strains of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* strain GG (formerly *Lactobacillus casei*) probably have the longest history of application as probiotics because of their health benefits. Currently used commercial probiotic products include *Lactobacillus* ssp., *Bifidobacterium* and even a few non-lactic acid bacteria.

SPECIFICITY OF YEAST

Saccharomyces boulardii, a patented yeast preparation, is the only yeast probiotic that has been proven effective in double-blind studies.[4] This yeast is used in many countries as both a preventive and therapeutic agent for diarrhoea and other GI disorders caused by the administration of antimicrobial agents. *Saccharomyces boulardii* possesses many properties that make it a potential probiotic agent, i.e. it survives transit through the GI tract, its temperature optimum is 37 °C, both *in vitro* and *in vivo,* it inhibits the growth of a number of microbial pathogens. However, *S. boulardii* belongs to the group of simple eukaryotic cells (such as fungi and algae) and, it thus differs from bacterial probiotics that are prokaryotes. Table 1 lists the main properties differentiating the yeast from the bacteria that account for the specificity of *S. boulardii* as a probiotic.

Yeast in microbial ecology

Commensal bacteria in the gut constitute a heterogenous microbial system containing approximately 10^{14} bacteria.[5] Yeast are a part of the residual microflora that makes up <0.1% of microbiota. Most yeast isolates from the GI tract are *Candida albicans,* although *Torulopsis glabratra* and *Candida tropicalis* are occasionally recovered.[6] Although yeast account for only a minority of the organisms making up the microbiota, their cell size is 10 times larger than that of bacteria (Figure 1) and they could represent a significant stearic hindrance for bacteria.

Microbial colonization of the human GI tract varies in number and species of bacteria as a function of environmental conditions.[5] The low pH of the stomach, ranging from 2.5 to 3.5, is destructive to most microbes; it grows up towards the distal part of the GI tract. While the pH rises towards the distal part of the GI tract, the presence of aggressive intestinal fluids (e.g. bile and pancreatic juice) and the short transit time in the duodenum, creates a hostile environment, and the duodenum thus contains relatively few microbes. Yeast are found in the stomach and colon. The presence of yeast in such different conditions can be explained by their resistance to pH variation (Table 1). In fact, while yeast grows well at pH 7–8, optimal growth is observed between pH 4.5 and 6.5. Most yeast can grow at pH 3.0, and some species can tolerate highly acidic conditions with a pH as low as

Table 1. Major differences between yeast and bacteria and their probiotic implications

	Bacteria	Yeast	Probiotic implication
Presence in human flora	99%	<1%	
Cell size	1 μm	10 μm	Stearic hindrance
Cell wall	Peptidoglycan, LPS (Gram-negative$^-$), LTA (Gram-positive)	Chitin, mannose (PPM, PLM), glucan	Immune response via TLRs, lectin receptors
Optimal growth conditions			
pH	6.5–7.5	4.5–6.5	Different sites of action in the GI tract
Temperature (°C)	10–80	20–30	
Resistance to antibiotics	No	Yes	Safety in combination with antibiotherapy
Transmission of genetic material (e.g. resistance to antibiotics)	Yes	No	

LPS, lipopolysaccharide; LTA, lipoteichoic acid; PPM, phosphopetidomannan; LPM, phospholipomannan; TLR, Toll-like receptor; GI, gastrointestinal.

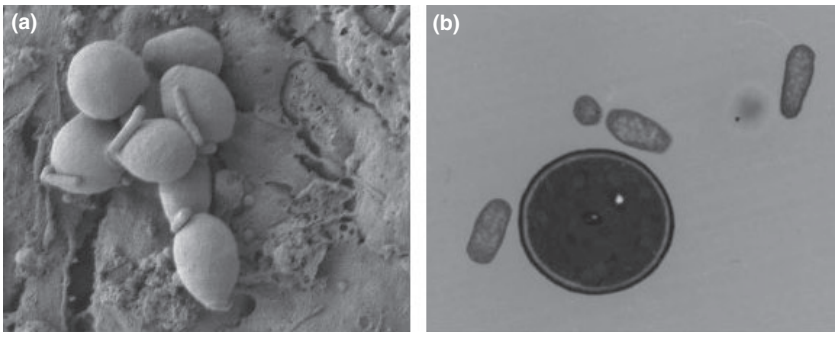

Figure 1. (a) Scanning electron micrographs of T84 cells exposed to *Saccharomyces boulardii* and *Salmonella typhimurium*. (b) Electron micrographs showing *S. boulardii* and *S. typhimurium*.

1.5. Yeast are thus good candidates as probiotics because probiotics entering the GI tract must be resistant to local stresses such as the presence of GI enzymes, bile salts, organic acids and considerable variations of pH and temperature.

Impact of antibiotics on yeast

The development of antimicrobial resistance by the pathogenic bacteria associated with antibiotic treatment has become an important public health problem. The natural resistance of yeast to antibacterial antibiotics is thus, a major argument for their use in antibiotic-treated patients. Antimicrobial resistance occurs both vertically (inherent or natural resistance of bacterial species or genus) and horizontally because of the transfer of genes between bacteria. The mammalian GI tract provides favourable conditions for the transfer of genetic material between many species of bacteria.[7] Resistance genes might be transferred not only between members of the resident gut flora, but also to and from transient bacterial probiotics. Recently, many investigators have speculated that commensal bacteria, including lactic bacteria, may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens. Genes conferring resistance to tetracycline, erythromycin and vancomycin have been detected and characterized in *Lactobacillus lactis*, Enterococci and, recently, in *Lactobacillus* species isolated from fermented meat and milk products and in strains used as probiotics (for review see Refs[8, 9]). The main threat associated with these bacteria is that they might transfer resistance genes to pathogenic bacteria. No such transfer of genetic material occurs between bacteria and yeast, making yeast safe for use during antibiotic treatment.

Cell wall components are determinants in the immune response

Substantial differences in the cell wall composition of bacteria and yeast have an impact on their antigenic responses. All bacteria contain a high-molecular weight sugar associated with protein that forms a rigid

structure called peptidoglycan. Gram-negative and Gram-positive bacteria differ in the lipid concentration of their cell wall. Gram-negative bacteria contain up to 20% lipids composed of lipopolysaccharide (LPS) while Gram-positive organisms have much fewer lipids in their cell walls but contain lipoteichoic acids (LTA).[10] The yeast cell wall consists of at least two layers. The outer layer contains a combination of mannose associated with either protein [phosphopetidomannan (PPM), commonly termed mannan] or lipid [phospholipomannan (PLM)]. The inner layer is composed of chitin and 1,3-β- and 1,6-β-glucan.[11] In living species, the first line of defence against microbial aggression is innate immunity.[12] Innate immunity relies on the recognition of pathogen-associated molecular pattern (PAMP) antigens by specific proteins referred to as pattern-recognition receptors (PRRs). Peptidoglycan, LPS and LTA, which are present in bacteria, and PLM, PPM and glycan, which are present in yeast, are all PAMPs and are recognized by different PPRs and thus can account for different responses of these micro-organisms as 'immunobiotics'.[12]

Specificity of *S. boulardii*

The non-pathogenic yeast *S. boulardii* was isolated from litchis in Indochina and is not a part of the autochthonous flora. It has been prescribed since mid-20th century, providing empirical evidence of its efficacy as an adjuvant agent for the treatment of diarrhoea and the prevention of AAD. Starting in the 1980s, research was conducted to evaluate the benefits of *S. boulardii* for the host organism and to determine its mechanisms of action. In particular, studies have investigated this yeast's effect in bacterial infections, its effects on the mucosa and, more recently, its immunomodulatory properties. *Saccharomyces boulardii* is the only yeast whose effect has been evaluated in double-blind clinical studies.

Saccharomyces boulardii was initially identified as a separate species of the hemiascomycete genus *Saccharomyces*.[13] In 1994, Cardinali and Martini[14] classified *S. boulardii* outside of *S. cerevisiae* species using comparative electrophoretic karyotyping and multivariate analysis of the polymorphism, observed in pulsed-field gel electrophoresis (PFGE). However, the rapid development of molecular phylogenetics in recent years, had led to changes in the classification of many yeast species.[15] Typing using four molecular techniques [species-specific polymerase chain reaction (PCR), randomly amplified polymorphic DNA–PCR, restriction fragment length polymorphic analysis of rDNA spacer region and PFGE] classified *S. boulardii* within the species *S. cerevisiae*.[16] Recently, Edwards-Ingram *et al.*[17] using comparative genomic hybridization for whole-genome analysis, also concluded that *S. cerevisiae* and *S. boulardii* are the members of the same species.

However, genetically *S. boulardii* differs from other *S. cerevisiae*. Hennequin *et al.*[18] identified a unique and specific microsatellite allele characterizing *S. boulardii* that distinguishes it from other strains of *S. cerevisiae*. Recently, pertinent characteristics of the *S. boulardii* genome such as trisomy of chromosome IX and altered copy number of individual genes have been revealed by comparative genome hybridization using oligonucleotide-based microarrays coupled with a rigorous statistical analysis.[17] Authors suggest that overexpression of gene related to protein synthesis and stress responses could be contributing to the increased growth rate and better survival of *S. boulardii* in acid pH.

In fact, metabolically and physiologically, *S. boulardii* differs considerably from *S. cerevisiae*, particularly, when it concerns as concerns growth yield and resistance to temperature and acidic stresses.[19] Whereas most *S. cerevisiae* strains grow and metabolize at a temperature of 30 °C, *S. boulardii* is a thermotolerant yeast that grows optimally at 37 °C, i.e. the physiological temperature of the host. Recent studies have demonstrated that *S. boulardii* appears to be more resistant than the *S. cerevisiae* strain W303 when exposed to a simulated gastric environment.[19]

However, the overexpression of genes in *S. boulardii* did not correlate with increased adherence to epithelial cells or transit through mouse gut.[17] Pharmacokinetic studies performed in man and rat, have shown that, after repeated administration, *S. boulardii* achieves steady-state concentrations in the colon within 3 days and is cleared from the stools 2–5 days after discontinuation.[20, 21]

Resistance to antibiotics

As *S. boulardii* is naturally resistant to antibiotics, it can be prescribed to patients receiving antibiotics.[22] Research on the administration of *S. boulardii* to patients suffering from recurrent *Clostridium difficile* infections has shown that the faecal yeast count is significantly higher in patients who do not relapse

compared to patients that do.[21] The efficiency of *S. boulardii* treatment thus appears correlated with the faecal yeast concentration.

Safety and packaging

Except for several sporadic reports of fungaemia, in patients with severe general or intestinal disease who had an indwelling catheter,[23] *S. boulardii* is considered to be a safe and well-tolerated treatment. The origin of these cases of fungaemia remains unclear, but is likely related to catheter colonization.[23, 24] Presence of such catheters is thus, a contraindication for the administration of *S. boulardii*.

Saccharomyces boulardii is administered in a lyophilized form, and is prepared, packaged and controlled as such. Therefore, lyophilized *S. boulardii* is clearly distinct from dietary probiotic products which contain diverse strains of micro-organisms and are used either in animals to improve zootechnical yields or in healthy humans (often in form of yogurt) to strengthen host physiology in the absence of any pathological context. *Saccharomyces boulardii* can be considered, an example of a 'probiotic drug'.

S. BOULARDII IN PLACEBO-CONTROLLED CLINICAL TRIALS

Antibiotic-associated diarrhoea

Antibiotic-associated diarrhoea is a common complication of antibiotic use. Surawicz *et al.*[25] evaluated the efficacy of *S. boulardii* administered during treatment and continued for 2 weeks, after the end of course in 180 hospitalized patients receiving antibiotics belonging to various classes. The incidence of diarrhoea was significantly reduced in patients receiving *S. boulardii* (10% vs. 22% in placebo, $P = 0.038$). The same authors carried out a similar study, focusing on β-lactam antibiotics and prolonging the follow-up period for 7 weeks, after the drug had been stopped.[26] *Saccharomyces boulardii* (at the dosage of 1 g/day) or placebo was administered to 193 patients from the beginning of antibiotic treatment and continued 3 days after the end of the course. In this study, *S. boulardii* mediated a significant preventive effect on the occurrence of diarrhoea (7% vs. 15%, $P = 0.02$). Similar results had been obtained by Adam *et al.*[27] in a study of 388 out-patients taking tetracycline or β-lactam antibiotics together with *S. boulardii* at a dosage of 200 mg/day. The frequency of diarrhoea was 18% in the placebo-treated group, compared to 5% in the *S. boulardii*-treated group ($P < 0.001$). A recent study investigated, the impact on AAD of *S. boulardii* in children with otitis media and/or respiratory tract infections.[28] Children received antibiotics plus 250 mg of *S. boulardii* ($n = 132$) or placebo ($n = 137$) orally, twice daily for the duration of antibiotic treatment. Analyses included data from 246 children and show that patients receiving *S. boulardii,* had a lower prevalence of diarrhoea than those who received the placebo (8% vs. 23%); RR, 0.3 (95% CI: 0.2–0.7); NNT, 7 (95% CI: 5–15). In a meta-analysis of probiotics for the prevention of AAD, D'Souza *et al.*[29] concluded that two types of probiotics (*S. boulardii* and S. *lactobacilli*) have the potential to be used in that situation. Nevertheless, *S. boulardii* is the probiotic that has been the most extensively studied with four large-scale placebo-controlled clinical studies showing a significant efficacy for preventing AAD (Table 2).

Clostridium difficile accounts for 20–25% of AAD in hospitalized patients and about 10% of AAD in community patients.[30] *Clostridium difficile* is responsible for 95% of pseudomembranous colitis. *Saccharomyces*

Table 2. Large-scale randomized trials of *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea (AAD)

Probiotics	Patients	*N*	Antibiotic treatment	Effect (% of diarrhoea) *S. boulardii* vs. placebo	*P*-value	Reference
S. boulardii	Hospitalized patients	180	Various	10 vs. 22	0.038	24
S. boulardii	Hospitalized patients	193	β-Lactam	7 vs. 15	0.02	25
S. boulardii	Out-patients	388	β-Lactam or cyclin	5 vs. 18	0.001	26
S. boulardii	Children in-patients and out-patients	246	Various	8 vs. 23	RR = 0.3	27

boulardii is the sole probiotic that has proven a significant efficacy in treating relapsing *C. difficile*-associated diarrhoea. In a randomized, placebo-controlled trial, McFarland *et al.*[31] evaluated the effect of *S. boulardii* (1 g/day for 28 days) and placebo as adjunctive therapy to metronidazole or vancomycin in 124 patients. It was the first episode of *C. difficile* infection in 64 cases and a relapse in 60 cases. In this study, after the administration of *S. boulardii,* the authors observed a 50% reduction of recurrences in patients who had previously experienced a first relapse of *C. difficile* infection. Surawicz *et al.*[32] obtained similar results, but only in patients receiving high-dose vancomycin.

Infectious diarrhoea

Traveller's diarrhoea

Traveller's diarrhoea is a well-known public health problem, particularly among travellers to developing countries. Enterotoxinogenic *Escherichia coli,* Shigellae and Salmonellae account for about 80% of cases with an identified pathogen in acute diarrhoea in travelers.[33] Kollaritsch *et al.*[34] evaluated the efficacy of *S. boulardii* for the prevention of diarrhoea in 1016 travellers visiting various countries in the world. The incidence of diarrhoea was 40% in patients receiving placebo, 34% in patients receiving *S. boulardii* 250 mg/day ($P = 0.019$) and 29% in patients receiving *S. boulardii* 1 g/day ($P < 0.005$). In a meta-analysis of probiotics for the prevention of traveller's diarrhoea analysing 12 different studies, McFarland[35] concluded that two probiotics, *S. boulardii* and a mixture of *L. acidophilus* and *Bifidobacterium bifidum,* had significant efficacy.

Acute diarrhoea in children

In children, infectious diarrhoea represents a public health problem and in the developing countries, several million children die of dehydration every year. In a meta-analysis assessing the efficacy of probiotics in the treatment and prevention of acute infectious diarrhoea, Szajewska *et al.*[36] have demonstrated that there is evidence of a clinically significant benefit of probiotics, *Lactobacillus* GG showing the most consistent effect. Since then, Kurugol *et al.*[37] have investigated the effect of *S. boulardii* in a double-blind randomized study involving 200 children. The duration of diarrhoea was significantly reduced (4.7 vs. 5.5 days, $P = 0.03$) as well as the number of days of hospitalization (2.9 vs. 3.9 days, $P < 0.001$). *Saccharomyces boulardii* has also been shown to be efficient, in reducing the number of children with prolonged diarrhoea (three of 44 vs. 12 of 44; RR 0.25; 95% CI: 0.1–0.8) in a double-blind, randomized study.[38] Finally, preliminary results suggest that, *S. boulardii* might be effective in preventing the occurrence of new episodes of diarrhoea in a 2-month long-term follow-up.[39]

Tube-feeding-associated diarrhoea

Diarrhoea is a common complication in critically ill patients receiving enteral nutrition. The addition of *S. boulardii* to nutrient supplements administered to patients receiving enteral nutrition decreased the incidence of diarrhoea. A 50% reduction in the number of treatment days with diarrhoea was reported in one study.[40] For patients suffering from moderate-to-severe burns receiving enteral nutrition, a reduction in the number of days with diarrhoea was accompanied by an increase in the mean number of calories tolerated per day.[41] In a multicentre, randomized, double-blind, placebo-controlled trial involving 128 critically ill tube-fed patients,[42] treatment with *S. boulardii* reduced the mean percentage of days with diarrhoea per feeding days from 19% to 14% (OR = 0.67, 95% CI: 0.50–0.90, $P = 0.0069$). The improvement of diarrhoea was more important in patients with high risk for diarrhoea (up to 42% vs. 25% in the whole study population).

AIDS

A randomized, double-blind trial covering 35 patients with AIDS-related diarrhoea showed the efficacy of *S. boulardii* 3 g/day given for 7 days in resolving diarrhoea. Sixty-one percentage of the patients were diarrhoea-free after 1 week of treatment with *S. boulardii* vs. 12% in the placebo group.[43]

Inflammatory bowel diseases

Three preliminary studies have evaluated the effect of *S. boulardii* in patients with IBD. A double-blind study of 20 patients suffering from Crohn's disease with moderate activity found that the addition of *S. boulardii* to conventional therapy with sulfasalazine or mesalazine (mesalamine) and corticosteroids significantly reduces bowel movements.[44] Similarly, a single-blind study of 32 patients with Crohn's disease of the ileum or colon

who had been in remission for ≥3 months[45] showed that 6-month maintenance therapy with mesalazine 500 mg twice daily plus *S. boulardii* 500 mg/day was significantly more effective in preventing relapse than mesalazine 500 mg three times daily ($P = 0.04$). Finally, an open pilot study evaluated 25 patients with a clinical flare-up of left-sided, mild-to-moderate ulcerative colitis receiving ≥3 months' maintenance therapy with mesalazine. The addition of *S. boulardii* 250 mg three times daily for 4 weeks to the mesalazine regimen resulted in therapeutic success according to Rachmilewitz's Clinical Activity Index (i.e. stool frequency, blood in stool) in 68% of the patients.[46] Further placebo-controlled studies are needed to support these preliminary results.

Irritable bowel syndrome

In a double-blind, placebo-controlled study in patients presenting with diarrhoea-predominant IBS,[47] *S. boulardii* treatment resulted in a decrease of the daily number of stools ($P < 0.05$) and an improvement of the consistency of the stools ($P < 0.05$).

EXPERIMENTAL EFFECT

Effect on enteric pathogens

Several studies using animal models or cell models indicated that *S. boulardii* may exert a beneficial effect against various enteric pathogens such as *C. difficile*, *Vibrio cholerae*, *Salmonella*, *Shigella* and *E. coli*. *Saccharomyces boulardii* appeared to act by two main mechanisms: (i) production of factors that neutralized bacterial toxins and (ii) modulation of the host cell signalling pathway implicated in proinflammatory response during bacterial infection (reviewed in Ref.[48] and Figure 2).

Neutralization of bacterial toxins

The antitoxin action of *S. boulardii* was demonstrated in cases of *C. difficile* infection. Toxin A, a 308 kDa protein, is a major virulence factor of *C. difficile*. Injection of toxin A into rodent intestines caused fluid secretion, increased mucosal permeability, mucosal damage and release inflammatory mediators. Oral administration of *S. boulardii* to rats before the addition of toxin A to the intestinal loop reduced toxin A-induced intestinal secretion and permeability.[49] Further investigation demonstrated that the addition of toxin A mixed with *S. boulardii*-filtered supernatant decreased toxin A-induced secretion. Two fractions were identified in *S. boulardii* supernatant: a fraction enriched in a 54 kDa serine protease that acted by proteolysis of both the toxin A and its receptor[50] and, another fraction (<10 kDa) that exerted an anti-inflammatory effect.[51]

Another antitoxin factor produced by *S. boulardii* was described in cases of cholera toxin (CT). Anatomo-

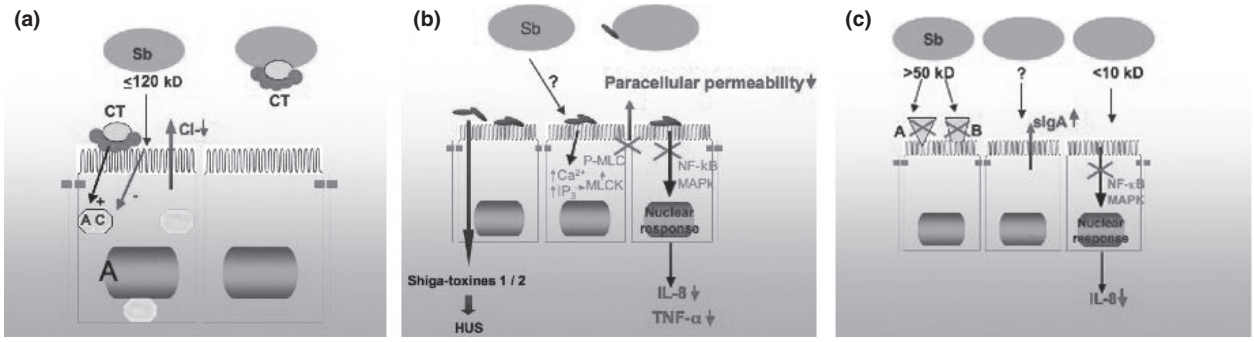

Figure 2. Proposed model for the mechanism of action of *Saccharomyces boulardii* against *Vibrio cholerae* (a), *Clostridium difficile* (b) and pathogenic *Escherichia coli* (EPEC and EHEC) infections (c). (Panel a) *Saccharomyces boulardii* produces a 120 kDa protein that exerts an effect on intestinal mucosa and inhibits cholera toxin (CT)-stimulated adenylate cyclase (AC) and chloride secretion. *Saccharomyces boulardii* also binds CT. (Panel b) *Saccharomyces boulardii* acts on intestinal mucosa and decreases phosphorylation of MLC implicated in the control of tight junctions as well as activation of MAPK and NF-κB implicated in the synthesis of proinflammatory cytokine IL-8 and TNF-α. (Panel c) *Saccharomyces boulardii* secretes a protease (>50 kDa) that lyses *C. difficile* toxins A and B and a protein (<10 kDa) that inhibits the signalling pathway implicated in IL-8 synthesis. *Saccharomyces boulardii* stimulates antitoxin A IgA production.

pathological studies on the small intestine of rats receiving *V. cholerae* alone and rats pre-treated for 5 days with *S. boulardii* have shown that the yeast prevents the morphological damage caused by *V. cholerae*.[52] Vidon *et al.*[53] demonstrated that the addition of *S. boulardii* to the intestinal loops of rats treated by CT decreased CT-induced fluid and sodium secretion by 50%. *In vitro* studies demonstrated that *S. boulardii* produces a 120 kDa protein that inhibits CT-stimulated chloride secretion by reducing the formation of cyclic AMP.[54, 55]

Saccharomyces boulardii also synthesized a phosphatase that can dephosphorylate endotoxins such as LPS from *E. coli* O55B5 and can partially inactivated its cytotoxic effects.[56] This mechanism may account for the protection afforded in cases of sepsis.

Modification of host cell signalling

Enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) shares a common pathogenic mechanism characterized by bacterial adhesion to the intestinal mucosa and subsequent changes in the integrity of tight-junction permeability and activation of signalling pathways (mitogen activated protein kinase (MAPK) and the transcription factor NF-κB) that stimulated IL-8 synthesis. *In vitro* studies have demonstrated that exposure of cells to *S. boulardii* before the addition of bacteria prevents the EPEC- and EHEC-induced decrease in transepithelial resistance and IL-8 secretion, suggesting that the yeast exerted a preventive effect.[57, 58] The observation that the yeast did not modify the number of adherent bacteria prompted research on the effects of *S. boulardii* on host cell signalling. The yeast has been shown to abolish phosphorylation of the myosin light chain (MLC) that is associated with a cytoskeletal protein involved in intercellular tight-junctions control. *Saccharomyces boulardii* also inhibits EPEC- or EHEC-induced NF-κB DNA-binding activity and activation of MAP kinases.[57, 58] TNF-α synthesis is controlled by MAP kinase and NF-κB. Recently, Dalmasso *et al.*[59] demonstrated that *S. boulardii* delays EHEC-induced apoptosis; this can be partially explained by the reduced TNF-α synthesis observed in the presence of yeast. Thus, *in vitro*, *S. boulardii* modified the signalling pathways implicated in proinflammatory cytokine synthesis.

Production of antitoxin factors as well as modification of proinflammatory responses of the host cell by *S. boulardii* do not exclude the possibility that other mechanisms may also account for the protective effect of *S. boulardii* in bacterial infection (Figure 2). *Saccharomyces boulardii* cell walls present binding properties for CT and EHEC.[52, 60] In cases of *C. difficile* infection, an inhibitory effect of *S. boulardii* on *C. difficile* adhesion has also been reported[61] as well as production of antitoxin IgA[62] (Figure 2c).

Effect on intestinal immune factors

Oral ingestion of *S. boulardii* causes an increase in secretory IgA and the secretory component in the rat small intestine. Buts *et al.*[63] found that growing rats which were given high doses of *S. boulardii* (0.5 mg/g body weight, three times a day) had an 80% increase in the secretory component of crypt cells and a 69% increase in villus cells. The mean secretory IgA level in the intestinal lumen was increased by 57%, and the polymeric immunoglobulin receptor concentration in crypt cells increased by 63% in *S. boulardii*-treated rats.

Trophic effect of *S. boulardii* on intestinal mucosa

Stimulation of brush-border membrane (BBM) enzymes by *S. boulardii* was first described in biopsies of human volunteers.[64] This study showed significant increases in the specific and total activity of sucrase-isomaltase (+82%), lactase (+77%) and maltase-glucoamylase (+75%) after 8 days of oral treatment by yeast compared to the baseline biopsies. These observations were confirmed in growing rats[65] and in two other studies conducted in rats after partial resection of the small bowel.[66, 67] At last, oral administration of *S. boulardii* improved dissaccharidase activities, enhanced the absorption of D-glucose coupled to Na+ by the symport glucose/Na+ and expression of the sodium-glucose cotransporter-1 (SLGT-1) in the brush-border of the remaining intestinal segments. *Saccharomyces boulardii* cells contain substantial amounts of polyamines (673 nmol/100 mg of lyophilized preparation of *S. boulardii*); in light of the well-known physiological effects of polyamines on cell maturation, enzyme expression and membrane transport mechanisms, Buts *et al.*[68] implicated polyamines as *S. boulardii*'s mediator of trophic effect. Recently, Schneider *et al.*[69] investigated the effect of *S. boulardii* administration on short-chain fatty acids (SFCA) faecal

concentration in patients on total enteral nutrition (TEN). They demonstrated that treatment with *S. boulardii* significantly increased total faecal SFCAs levels in TEN patients (150 ± 27.2 vs. 107 ± 18.2 mmol/kg), whereas no modification of SFCA was observed in controls. At the end of treatment with *S. boulardii* these TEN patients had higher faecal butyrate (16.0 ± 4.4 vs. 10.1 ± 2.9). In these patients *S. boulardii* did not modify faecal flora. Total SCFAs remained high 9 days after treatment was discontinued. The authors conclude from this study that *S. boulardii*-induced increase of faecal SCFAs concentration (especially butyrate) may explain the preventive effect of this yeast on TEN-induced diarrhoea.

Anti-inflammatory effect of *S. boulardii*

The beneficial effect of *S. boulardii* on intestinal inflammation was investigated in two murine models of colitis: $CD45RB^{hi}$ $CD4^+$ T cell-restored *SCID* mice (chronic inflammation); and 2,4,6-trinitrobenzoic sulfonic acid (TNBS)-treated mice (acute colitis).[70, 71] In both models, *S. boulardii* afforded protection from histological damage, suppressed NF-κB activation, and inhibited proinflammatory cytokine gene expression.[72, 73]

Several molecules have been hypothesized to play a role in this anti-inflammatory effect of *S. boulardii*. A small (<1 kDa) heat-stable and water-soluble anti-inflammatory molecule termed *Saccharomyces* anti-inflammatory factor (SAIF) has been identified in the yeast supernatant.[74] Butyrate has been shown to inhibit the inflammatory response via inhibition of NF-κB[75] and, butyric acid synthesis is increased in patients on enteral nutrition receiving *S. boulardii*.[69] Another study has suggested that *S. boulardii* stimulates PPAR-γ expression and reduces the response of human colon cells to proinflammatory cytokines.[76] Finally, administration of *S. boulardii* to rats with castor oil-induced diarrhoea modulates expression of the iNOS molecule that has also been implicated in the control of acute colitis.[77]

Saccharomyces boulardii has been shown to modify the migratory behaviour of lymphocytes.[73] This effect was described in the chronic model of IBD based on injection of naive $CD4^+$ $CD45RB^{hi}$ T lymphocytes that leads to the development of severe colitis characterized by infiltration of pathogenic IFN-γ-producing $CD4^+$ T cells within the colon mucosa.[70] In animals treated with *S. boulardii,* the inhibition of inflammation was correlated with a decrease of IFN-γ-producing $CD4^+$ T cells within the colonic mucosa and an enrichment of IFN-γ-producing T cells in the mesenteric lymph nodes (mLN). Further research demonstrated that *S. boulardii* supernatant modifies the capacity of endothelial cells to adhere to leucocytes, allowing better cell rolling and adhesion. This last finding suggests, new pathways of investigation to improve our understanding of the action of *S. boulardii*. Identification of molecule(s) that inhibit(s) NF-κB activation and modify(ies) T lymphocyte cell rolling is primordial.

CONCLUSION

The antidiarrhoeal effect of lyophilized *S. boulardii* has been investigated in several forms of diarrhoeal diseases. The clinical efficacy of the yeast has been clearly demonstrated for both the prevention of AAD and the treatment of recurrent *C. difficile* disease. The superiority of probiotic yeast over probiotic bacteria in these indications is probably because of the natural resistance of yeast to antibacterial antibiotics, which leaves intact their viability and probiotic properties.

Data on acute gastroenteritis and on traveller's diarrhoea are accumulating. The clinical relevance of *S. boulardii* in inflammatory bowel diseases needs further investigation.

Investigations designed to elucidate the mechanisms of action of *S. boulardii* have demonstrated the existence of additional mechanisms: release *in vivo* of substances that inhibit certain bacterial toxins and/or their pathogenic effects; trophic effects; antisecretory activity and immunostimulatory effects on the intestinal mucosa. The recent discovery of an anti-inflammatory effect opens the door to new clinical applications.

In conclusion, the activity of probiotics is strain-dependent, and in this context *S. boulardii* is the only yeast probiotic presenting its own specificity.

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