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## Technical Report

# *Saccharomyces boulardii* in Gastrointestinal Related Disorders

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## *Saccharomyces boulardii* in Gastrointestinal Related Disorders

*Saccharomyces boulardii* is a non-pathogenic yeast widely used as a probiotic agent in many countries. Originally isolated from the litchi fruit (*Litchi chinensis*) by Dr. Henri Boulard in 1923, *S. boulardii* has since become a well known preventative and treatment for a number of gastrointestinal disorders including *Clostridium difficile* infection, antibiotic-associated diarrhea, and inflammatory bowel diseases. *S. boulardii* is genetically resistant to all antibacterial antibiotics,<sup>1,2</sup> making it a practical and cost effective addition to typical antibiotic therapies which destroy beneficial bacteria within the gut. In most cases, *S. boulardii* takes approximately three days to achieve viable concentrations within the gut, and is cleared within 2-5 days after oral supplementation.<sup>1</sup> *S. boulardii* has been safely used in many patients including children and the elderly.

### Clinical Use and Associated Mode of Action

As the health benefits of *S. boulardii* become more well known, researchers are beginning to take a closer look at how this probiotic yeast interacts with its host. A number of in vitro and animal studies have been published examining some of the mechanisms by which *S. boulardii* acts. To date *S. boulardii* has been found to act in two principle ways: first it inhibits some bacterial toxins or their effects; and secondly it has direct effects on the intestinal mucosa.<sup>1,2,3,4,5,6</sup> In this paper, we will further explore these two mechanisms and detail the clinical data available for *S. boulardii*.

### Antibiotic Associated Diarrhea

Antibiotic therapy is widely prescribed in the United States for an array of conditions. It is well known that antibiotics eradicate beneficial bacteria which populate the intestines and colon, making room for more harmful bacteria to flourish.<sup>7</sup> Without these beneficial bacteria, the ecosystem of the gut becomes disrupted, resulting in dysbiosis. Dysbiosis can lead to antibiotic associated diarrhea (AAD), a condition that is often difficult to treat and can even become life threatening in severe cases. *S. boulardii* has a history of use for the treatment of AAD and continues to be a popular alternative to typical drug treatments. In fact, the strain CBS 5926 has been approved by the German Commission E for treatment of acute diarrhea, prophylactic and symptomatic treatment of diarrhea during travel, and for diarrhea occurring during tube feeding.

In a double-blind placebo-controlled study examining the preventative effects of *S. boulardii* on AAD in adults, 78 patients were given antibiotics plus placebo, while 73 patients were given antibiotics plus *S. boulardii* (10 billion CFU/day). In the placebo group, 7 patients (9%) developed diarrhea, while only one (1.4%) patient in the *S. boulardii* group developed diarrhea.<sup>8</sup> Another double-blind placebo-controlled study which involved 246 children receiving antibiotic therapy for otitis media and/or respiratory tract infections found patients receiving 10 billion CFU/day of *S. boulardii* had less occurrences of AAD (4 of 119) compared to those who received placebo (22 of 127).<sup>9</sup> Surawicz et al. also reported a decrease in AAD among adults receiving 10 billion CFU/day of *S. boulardii* (9.5% versus 21.8% in the placebo group).<sup>10</sup> In a large double-blind study involving 20 different medical centers, 388 patients (age >15 years) with AAD were given either placebo or 20 billion CFU/day of *S. boulardii* along with antibiotics. Those receiving the *S. boulardii* had fewer disturbances in intestinal transit (4.52% compared to 17.46%).<sup>11</sup> These same data have been confirmed in numerous other trials.<sup>12,13,14,15</sup>

While any antibiotic can cause AAD, some increase the risk more than others. Ampicillin, clindamycin, cephalosporins, erythromycins, quinolones, and tetracyclines are high on the list of AAD causing antibiotics.<sup>16</sup> Clinicians should consider recommending 10-20 billion CFU/day of *S. boulardii* when prescribing any of these antibiotics which increase the risk of AAD.

### *Clostridium difficile*

*Clostridium difficile* is a bacteria that is known to flourish during antibiotic use and is responsible for up to 20% of antibiotic associated diarrhea cases.<sup>17,18</sup> It is also known to be one of the primary causes of pseudomembranous colitis, an infection of the colon. *C. difficile* can be very difficult to treat, approximately 80% of the population responds to typical antibiotic treatment while the other 20% do not and may experience recurrent *C. difficile* infections for many years.<sup>19</sup> Those who have had one recurrent *C. difficile* infection are 63% more likely to have future recurrent infections.<sup>20</sup> While few clinical trials have taken place in which the efficacy of *S. boulardii* treatment on *C. difficile* infection is the primary outcome, this is often reported in studies involving antibiotic associated diarrhea.

Thirteen patients between the ages of 40-81 with an active *C. difficile* infection were given standard antibiotic therapy for 5 days, on the 5<sup>th</sup> day, the addition of *S. boulardii* (5 billion CFU/ twice daily) for 30 days was included. After the treatment was completed, 11 patients were retested for *C. difficile*. Of the 11 patients, 55% were culture negative and 82% cytotoxin negative.<sup>20</sup> In another study, 19 children with an active *C. difficile* infection received 250 mg *S. boulardii* two to four times per day (depending on age) for 15 days. The eradication of *C. difficile* from stools was found in 73% of patients after one month and toxin B was cleared within 15 days in 85% of patients.<sup>21</sup> In a double-blind placebo-controlled study, 124 patients with recurrent or initial *C. difficile* associated disease (CDD) were given antibiotics with the addition of *S. boulardii* (20 billion CFU/day) or placebo for 4 weeks. In patients with recurrent *C. difficile* infections, *S. boulardii* reduced the risk of multi-recurrence (34.6% versus 64.7%).<sup>22</sup> Other studies and meta-analysis have also reported similar findings.<sup>23,9,24,10,12</sup>

*Saccharomyces boulardii* interacts with *C. difficile* on multiple levels and has been studied in animals, humans, and in vitro studies. *C. difficile* discharges two exotoxins, known as toxin A and toxin B. These toxins trigger immune cells to release inflammatory mediators (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8)<sup>25</sup> and wreak havoc on the gastrointestinal tract, leading to diarrhea, inflammation, and other symptoms observed during infection. In vivo, *S. boulardii* has been found to release a 54 kDa protease, which digests toxin A and its receptor on the brush border membrane.<sup>5,26,27</sup> In rat ileal loops, the 54 kDa protease was found to inhibit the binding of toxin A to brush border membranes, reduce hydroelectrolytic secretions, partially inhibit the intestinal permeability to manitol, and inhibit tissue damage caused by toxin A.<sup>27,26</sup> In vitro data suggests the 54 kDa protease may also have enzymatic activity against toxin B and digest its receptor on the brush border membrane as well.<sup>5</sup>

In vitro, toxin A stimulates ERK and p38 MAP kinases.<sup>3,25</sup> MAP (mitogen-activated protein) kinases are responsible for regulating cellular activities like gene expression, mitosis, and cell survival. In vitro (human colonocyte cells), *S. boulardii* was found to inhibit the activation of ERK 1/2 and JNK, IL-8, and IL-1 $\beta$ .<sup>3</sup> *S. boulardii* did not inhibit p38 activation. In a mouse ileal loop model, *S. boulardii* also inhibited ERK 1/2 activation.<sup>3</sup> This inhibition of MAP kinase activity is thought to play a significant role in how *S. boulardii* limits *C. difficile* related symptoms.

*S. boulardii* also stimulates an immune response against toxin A in mice. *S. boulardii* stimulated the secretion of IgA and IgM antitoxin A in the serum.<sup>28</sup> Rats receiving 0.5 mg/g body weight *S. boulardii*, had a mean increase of secretory IgA by 56.9% compared to those receiving placebo.<sup>29</sup> This action may be a result of the polyamines, spermine and spermidine, found on lyophilized *S. boulardii*. Rats administered spermine and spermidine also had increased IgA secretion and enzyme activity.<sup>30</sup>

Tasteyre et al demonstrated that *S. boulardii* inhibited the attachment of *C. difficile* to intestinal cells in certain conditions.<sup>2,4</sup> Cell wall fractions of *S. boulardii* were shown to prevent *C. difficile* from attaching to Vero cells (monkey kidney epithelial cells). Tasteyre et al conclude that *S. boulardii* could inhibit the attachment of *C. difficile* to cells and suggested that a cell wall proteolytic enzyme associated with *S. boulardii* may account for the ability to prevent *C. difficile* from attaching to the cell surface receptors.<sup>4</sup>

### Traveler's Diarrhea

Traveling to a foreign country, or even a different state, where diverse foods are consumed and different sanitary methods may be practiced can alter the gut flora and lead to dysbiosis. Worldwide, approximately 10 million people each year develop traveler's diarrhea. Those who may be immuno-compromised, children, and the elderly are most affected. *S. boulardii* has been successfully used in trials to reduce the incidence of traveler's diarrhea and may help millions of traveler's each year eliminate this unwanted condition.

In a study comparing different non-antibiotic preparations (*L. acidophilus*, Dodecoral, Noventerol, and *S. cerevisiae* strain Hansen CBS 5926) for the prevention of traveler's diarrhea, *S. cerevisiae* was found to exert prophylactic effects on traveler's diarrhea, while the other preparations had little to no effect. Of the participants in the placebo group (n=406), 42.6% experienced diarrhea. Only 33.6% of participants receiving 250 mg/day of *S. cerevisiae* (n=424) and 31.8% receiving 500 mg/day of *S. cerevisiae* (n=399) experienced diarrhea.<sup>31</sup> In a similar study by Kollaritsch et al, 1016 people traveling to distant regions were given either placebo, 250 mg/day of *S. boulardii*, or 1 g/day of *S. boulardii*. Those receiving the *S. boulardii* treatments reported fewer incidences of diarrhea (39.1%, 34.4%, and 28.7% respectively).<sup>32</sup>

### Acute Diarrhea

Worldwide, acute diarrhea is responsible for over 2 million deaths each year.<sup>33</sup> Acute diarrhea is defined as having 3 or more loose bowel movements in 24 hours and lasting less than 3-4 weeks; it can be caused by a number of factors including bacteria, parasites, drugs, allergies, and other gastrointestinal conditions. Typical treatments include hydration therapy, over-the-counter (OTC) anti-diarrheal medications, and the "wait and see" method. Billoo et al. reported 50 children (2 months – 2 years) with acute diarrhea given 250 mg of *S. boulardii* twice per day along with oral rehydration salt and nutritional support had a reduced frequency of stools and duration of diarrhea compared to those receiving placebo with oral rehydration salt and nutritional support (n=50).<sup>33</sup> Cetina-Sauri and Sierra Basto reported similar results in children receiving *S. boulardii*, oral rehydration salt and nutritional support.<sup>34</sup> A study conducted by Höchter et al. found a significance in days with diarrhea in an adult population receiving *S. boulardii* monotherapy for acute diarrhea.<sup>35</sup> *S. boulardii* used as a monotherapy and as an adjunct to oral rehydration therapy, may provide a strong alternative treatment for those suffering from acute diarrhea.

### Enteral Feeding

It is estimated that approximately 68% of those undergoing enteral feeding develop diarrhea.<sup>36</sup> Changes in the intestinal flora are suggested to be the primary factor.<sup>37,38</sup> In one double-blind placebo-controlled study, 128 critically ill tube-fed patients were given either 500 mg of *S. boulardii* four times per day or placebo (64 patients in each group). Receiving the *S. boulardii* reduced the average number of days with diarrhea from 18.9% to 14.2%.<sup>39</sup> Another double-blind placebo-controlled trial including enterally fed patients, reported those receiving *S. boulardii* experienced approximately a 50% reduction in days with diarrhea compared to placebo (8.7% and 16.9%, respectively).<sup>40</sup> These studies suggest supplementation with *S. boulardii* may be beneficial in restoring the intestinal flora balance and reducing enteral tube feeding induced diarrhea.

### Inflammatory Bowel Disorders

Inflammatory bowel disease, Crohn's disease, irritable bowel syndrome (IBS), and ulcerative colitis are inflammatory diseases of the intestines and colon. These diseases are becoming more prevalent in the United States and have no clear etiology. Many of these diseases have high relapse rates and are characterized by weight loss, abdominal pain, diarrhea, and cramping. Modern drug treatments for these disorders focus on sustaining remission by using corticosteroids and aminosalicylates.<sup>41</sup> Only a small handful of human clinical trials exist for the use of *S. boulardii* in irritable bowel diseases; however, results of these trials are promising.

In a randomized trial, 32 patients with Crohn's disease in remission were divided into two groups. Group A (n=16) received mesalazine two capsules (Pentasa 500 mg) three times per day, while group B (n=16) received two capsules (Pentasa 500 mg) twice a day and *S. boulardii* 500 mg two capsules once per day. After 6 months of treatment, patients in group B had fewer relapses than those in group A (1 of 16 and 6 of 16, respectively). It was also noted that those receiving the *S. boulardii* reported improvements in the Crohn's disease activity index (abdominal pain, general well being, etc) as well as episodes of diarrhea.<sup>42</sup> Plein and Hotz conducted a randomized double-blind placebo-controlled pilot study consisting of 20 Crohn's disease patients. For two weeks all patients received standard treatment (Azulfidine<sup>®</sup>, Colopleon<sup>®</sup>, Salofalk<sup>®</sup>, Calversal<sup>®</sup>, Imurek<sup>®</sup>, Prednisone or a combination of above treatments) with 250 mg *S. boulardii* three times per day. A reduction in the number of bowel movements was noted compared to baseline data. Patients were then randomized to receive placebo and standard treatment (n=7) or *S. boulardii* 250 mg three times per day and standard treatment (n=10) for seven weeks. Patients receiving *S. boulardii* reported a significant reduction in the number of bowel movements.<sup>43</sup>

A pilot trial of 25 patients with mild to moderate flare up of ulcerative colitis was conducted. Patients receiving mesalazine treatment were also given 250 mg *S. boulardii* three times per day for 4 weeks. Of the 24 patients who completed the trial, 17 patients had achieved clinical remission.<sup>44</sup> In a double-blind placebo-controlled trial, 34 patients with irritable bowel syndrome were given *S. boulardii* (n=16) or placebo (n=18) for one month. Those receiving *S. boulardii* reported statistically significant improvements in symptoms (frequency of bowel movements, consistency, etc) compared to those receiving placebo.<sup>45</sup> While the use of *S. boulardii* for inflammatory bowel diseases is relatively new, these studies suggest *S. boulardii* may be an important adjunct to current therapies.

Animal and in vitro studies have been conducted to determine possible mechanisms by which *S. boulardii* acts on inflammatory bowel diseases. In a study done in mice induced with irritable bowel disease, *S. boulardii* altered the behavior of pro-inflammatory T cells. After administration of *S. boulardii*, T-lymphocyte cells migrated into mesenteric lymph nodes, thereby inhibiting the migration of IFN- $\gamma$  producing T-cells into the colon. The authors suggest this behavior may implicate that *S. boulardii* secretes molecules that modify the adhesiveness of endothelial cells from mesenteric lymph nodes toward T-lymphocytes. In this same study, NF-kB was found to be activated in mice with induced irritable bowel disease. *S. boulardii* was able to inhibit NF-kB activity. Furthermore, *S. boulardii* decreased the expression levels of the cytokines TNF- $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$ , IL-6 in a dose dependant manner.<sup>41</sup> An in vitro study supports these same findings and found that *S. boulardii* secretes an anti-inflammatory factor that is less than 1 kDa. This secretion blocks NF-kB activation and NF-kB influenced IL-8 gene expression in both intestinal epithelial cells and monocytes, preserves the degradation of I $\kappa$ B $\alpha$  (responsible for inhibiting NF-kB), and reduced NF-kB DNA-binding in monocytes.<sup>46</sup>

#### Butyrate production by *S. boulardii* in Inflammatory Bowel Disorders

Probiotics produce short chain fatty acids (SCFA) during the fermentation of undigested complex carbohydrates and proteins. Short chain fatty acids (butyrate, acetate, and propionate) provide fuel to the colon epithelial cells and other tissues and play a role in colonic re-absorption of water and other electrolytes.<sup>6,2</sup> It is estimated that colon epithelial cells derive 70% of their energy from the oxidation of butyrate and that butyrate plays a beneficial role in modulating chronic inflammation of intestinal mucosa.<sup>6</sup> Decreased efficiency in oxidizing butyrate within colonic epithelial cells is associated with some cases of ulcerative colitis.<sup>47 48</sup>

The interaction between butyrate and inflammatory bowel disorders has been examined in vivo and in vitro. Epithelial cells isolated from the descending colon of patients with ulcerative colitis, Crohn's disease, diverticulitis, and cancer were incubated with butyrate. Compared to control-treated cells, butyrate increased protein synthesis in ulcerative colitis cells, suggesting that butyrate may increase protein production and promote healing in epithelial cells of ulcerative colitis patients.<sup>49</sup> Segain et al. isolated lamina propria cells and peripheral blood mononuclear cells from 17 patients with Crohn's disease. The cells were cultured with and without butyrate. Results showed butyrate inhibited the degradation of I $\kappa$ B $\alpha$ , preventing NF-kB activation. Butyrate also decreased TNF- $\alpha$  production, as well as IL-6, TNF- $\beta$  and IL-1 $\beta$ , and decreased pro-inflammatory cytokine mRNA expression.<sup>50</sup> A reduction in NF-kB and IL-1 $\beta$  was seen in 7 of 9 patients with Crohn's disease receiving 4 grams of butyrate per day for 8 weeks.<sup>51</sup> Ulcerative colitis patients receiving butyrate also had a decrease in mucosal inflammation due to a reduction of NF-kB in lamina propria macrophages.<sup>52</sup>

The use of *Saccharomyces boulardii* on short chain fatty acid production, butyrate in particular, has had mixed but promising results. In a study involving 10 patients on long-term total enteral feeding, *S. boulardii* (500 mg twice per day for 6 days) was found to increase total fecal SCFA levels, especially butyrate.<sup>53</sup> In a porcine model, *S. boulardii* was able to partially restore production rates of SCFA after clindamycin administration, in particular acetate and propionate, but not butyrate.<sup>54</sup> These studies suggest the production of butyrate by *S. boulardii* may potentially play a key role in the management of inflammatory bowel diseases, further research is warranted.

#### *Escherichia coli*

*Escherichia coli* is a bacteria found in the lower intestines and colon. When kept in balance, *E. coli* can be beneficial to the body, assisting with vitamin K production and waste processing; however, an overgrowth of *E. coli* can cause illnesses such as diarrhea, urinary tract infections, and other gastrointestinal infections. The pathology of *E. coli* is both multi-factorial and destructive. *E. coli* (enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC)) adhere to the gastrointestinal tract, causing a disruption/modification of the tight-junctions, in turn leading to an increase in the intestinal permeability and diminished barrier function.<sup>55,56,57,58</sup> The phosphorylation of myosin light chain, found to be provoked by an EPEC infection, has been implicated in the disturbance of the intestinal epithelial barrier function.<sup>58</sup> Following this initial disruption, a transmigration of polymorphonuclear leukocytes occurs<sup>59</sup>, pro-inflammatory cytokines are synthesized,<sup>60,56,61</sup> and MAP kinases (ERK1/2, p38, and JNK) are activated,<sup>60,62</sup> ultimately leading to cell death.<sup>61</sup>

The effects of *S. boulardii* were examined on EHEC in T84 cells (intestinal epithelial cells) in vitro. Cells were incubated with *S. boulardii* and EHEC. While *S. boulardii* did not have an effect on EHEC growth or adhesion, *S. boulardii* did inhibit EHEC induced myosin light chain phosphorylation. Furthermore, pre-incubation of cells with *S. boulardii* before the induced EHEC infection were found to inhibit both NF- $\kappa$ B DNA binding and phosphorylation and degradation of I $\kappa$ B $\alpha$ . *S. boulardii* also inhibited the activation of three (ERK 1/2, p38, and JNK) MAP kinases.<sup>56</sup> In another study, T84 cells were infected with EHEC and *S. boulardii* or EHEC alone. Those cells infected with EHEC and *S. boulardii* had significantly less TNF- $\alpha$  and related apoptosis than those not inoculated with *S. boulardii*.<sup>61</sup>

The outer membrane of *S. boulardii* is rich in mannose, allowing pathogens such as enteropathogenic *E. coli*, salmonellae and other type 1 pili (mannose-binding fibers) containing pathogens to bind to this mannose rich membrane. This binding action prevents *E. coli* and other harmful bacteria from adhering to intestinal cells. This action may also be beneficial for other probiotic species as there is less competition for colonization. Gedek examined if other enteric bacteria such as enterohaemorrhagic *E. coli* and a mutant of *Salmonella typhimurium* (a multiple antibiotic resistant strain) have the ability to bind mannose. It was found that some EHEC isolates (assumed to be the ones capable of producing pili or other mannose-sensitive adhesions) and the mutant *Salmonella typhimurium* adhere to this mannose rich membrane.<sup>63</sup> In a study examining the effects of *S. boulardii* on *E. coli* status in children, it was found that *S. boulardii* (5 billion CFU/once a day) supplementation for 5 days, dramatically reduced the number of *E. coli* colonies from 384,625 (+/-445,744) to 6,283 (+/-20,283). The authors suggest *S. boulardii* may be a beneficial agent in the prevention of urinary tract infections in pediatric children, primarily caused by ascending microorganisms from the gastrointestinal tract.<sup>64</sup>

*Saccharomyces boulardii* has been shown to be an effective agent against *E.coli*, *C. difficile*, and a number of other gastrointestinal conditions through multiple mechanisms. The use of *S. boulardii* against other pathogens such as *Vibrio cholerae*<sup>65</sup>, *Giardia lamblia*<sup>66</sup>, *Shigella flexneri*, and *Salmonella typhimurium*<sup>67</sup>, and *Candida albicans*<sup>68,69,70</sup> have also been demonstrated, likely through the previously discussed mechanisms.

### Trophic Properties

Beyond its numerous effects on various pathogens and gastrointestinal conditions, *Saccharomyces boulardii* also has a number of other trophic properties that are beneficial to its host. *S. boulardii* has been found to increase several brush border microvillous enzymes in humans, including lactase,  $\alpha$ -glucosidases,  $\beta$ -galactosidase, alkaline phosphatase, sucrase-

isomaltase, and maltase-glucoamylase.<sup>71,1,72</sup> These enzymes play a role in nutrient absorption and digestion in humans. Another action of *S. boulardii* is to secrete sucrase, which may play a beneficial role in patients with sucrase-isomaltase deficiency.<sup>73</sup>

### Safety

The administration of *S. boulardii* to a diverse patient population has been both effective and safe. Rare reports of fungemia (a yeast or fungal infection of the blood) have been reported in those who are immune compromised, but have not been reported in those who are otherwise “healthy”.<sup>74</sup> To date, the longest published clinical trial using *S. boulardii* was a study involving Crohn’s disease patients for 6 months.<sup>42</sup> No adverse effects were reported in any of the patients. While typical *S. boulardii* treatment calls for short-term use, there are some conditions in which long-term *S. boulardii* treatment is called for.

### Conclusion

*Saccharomyces boulardii* is a probiotic yeast that has been used successfully in many different gastrointestinal disorders and bacterial infections. There are numerous clinical trials showing its ability to reduce or prevent anti-biotic associated diarrhea, abolish or reduce symptoms associated with *C. difficile*, and lessen or prevent diarrhea associated with travel and tube feeding. Studies have also successfully used *Saccharomyces boulardii* in inflammatory bowel disorders such as Crohn’s disease, irritable bowel disease, and colitis. From these studies we have learned *Saccharomyces boulardii* has multiple effects on its host including its ability to inhibit some bacterial toxins or their effects and having direct effects on the intestinal mucosa. Research suggests individuals taking antibiotics or suffering from an inflammatory bowel disease take 10-20 billion CFU’s of *Saccharomyces boulardii* per day (see attached chart for a summary of dosing reviewed in this paper). While more research is needed to further understand some of the mechanisms by which *S. boulardii* acts in humans, clinical data to date supports its use in gastrointestinal related conditions.



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