Saccharomyces boulardii effects on gastrointestinal diseases

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Abstract

Health benefits attributed to probiotics have been described for decades. They include the treatment and the prevention of gastrointestinal diseases, vaginal and urinary infections and allergies. *Saccharomyces boulardii*, a species of yeast widely distributed, has been described as a biotherapeutic agent since several clinical trials displayed its beneficial effects in the prevention and the treatment of intestinal infections and in the maintenance of inflammatory bowel disease. All these diseases are characterized by acute diarrhoea. Administration of the yeast in combination or not with an antibiotherapy has shown to decrease significantly the duration and the frequency of diarrhoea. Experimental studies elucidated partially the molecular mechanisms triggered to improve the host health. The discovery of its anti-inflammatory and immuno-modulatory activities in correlation with the advances in the understanding of mucosal immunology opens a new field of perspectives in *S. boulardii* therapeutic applications.

Introduction

The intestine contains a complex and dynamic microflora including more than 2000 micro-organism species coexisting in a complex equilibrium with the host. This microflora has various effects including metabolic activities, trophic effects on the intestinal epithelium, interactions with the host immune system (Guarner and Malagelada, 2003) and acts as a barrier to prevent colonization by opportunistic and pathogenic micro-organisms (Vollaard and Clasener, 1994). The immune system and particularly the gut-associated lymphoid tissues (GALT) provides the host with protective mechanisms against pathogen invasion across the intestinal mucosal surface (Acheson and Luccioli, 2004). However, disequilibrium in the gut microflora ecology and in the immune response could induce gastrointestinal diseases.

The term “probiotic” has been firstly defined by Fuller as “a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance” (Fuller, 1989). This definition has been extended to health and probiotics were redefined as “live micro-organisms that, when administered in adequate amounts, confer a health benefit to the host” (FAO/WHO, 2001). *Saccharomyces Boulardii*, isolated from litchi fruit by Henri Boulard in the 1920s, belongs to the *Saccharomyces genus* which is commonly used in several food processes including beverages and bred fermentation. This yeast is frequently prescribed in a lyophilized form as a biotherapeutic agent (Elmer et al., 1996; Klein et al., 1993). Indeed, controlled clinical trials have shown that oral administration of *S. boulardii* could treat or prevent gastrointestinal diseases such as antibiotic-associated diarrhoea (Kotowska et al., 2005), recurrent *Clostridium difficile*-associated diseases (McFarland, 2006), traveller’s diarrhoea (McFarland, 2007), children acute diarrhoea (Htwe et al., 2008), enteral tube feeding-associated diarrhoea (Bleichner et al., 1997), AIDS-associated diarrhoea (Saint-Marc et al., 1991), intestinal bowel disease such as Crohn’s disease and ulcerative colitis (Guslandi et al., 2003; Guslandi et al., 2000) and irritable bowel syndrome (Maupas et al., 1983).

S. boulardii and *S. cerevisiae* are members of the same species (Edwards-Ingram et al., 2007) but they differ genetically, metabolically and physiologically (Fietto et al., 2004; Hennequin et al., 2001). *S. boulardii* is characterized by a specific microsatellite allele (Hennequin et al., 2001) and recent studies showed that *S. boulardii* genome presents trisomy of the chromosome 9, altered copy numbers of genes potentially contributing to the increased growth rate and a better survival in acidic environment (Edwards-Ingram et al., 2007). In addition to a better resistance to acidic stress, *S. boulardii* grows faster than *S. cerevisiae* at 37°C (Fietto et al., 2004). Pharmacokinetic studies have shown that after oral administration of lyophilized *S. boulardii*, the steady-state concentrations are achieved in the colon within 3 days, and the yeast are cleared from the stools within 2-5 days after discontinuation (Blehaut et al., 1989). *S. boulardii* displays important characteristics allowing a micro-organism to transit through the gastrointestinal tract and to be used as a probiotic. During the intestinal transit, *S. boulardii* interacts with resident microflora and intestinal mucosa. Moreover, experimental studies displayed that *S. boulardii* induces a protection against enteric pathogens (Czerucka and Rampal, 2002; Mumy et al., 2007), modulates the host immune response (Ozkan et al., 2007; Rodrigues et al., 2000), decreases inflammation (Lee et al., 2005; Sougioultzis et al., 2006) and hydroelectrolytic secretions (Czerucka and Rampal, 1999), inhibits bacterial toxins (Castagliuolo et al., 1999; Tasteyre et al., 2002) and enhances trophic factors such as brush border membrane enzymes and nutrient transporters (Buts et al., 1986; Buts et al., 1994).

The aim of this review is to summarize the current knowledge about the beneficial effects of *S. boulardii* on gastrointestinal diseases. Firstly, clinical trials will be succinctly reviewed. Then, the experimental studies which have lead to a better understanding of the mechanisms used by the yeast to prevent and/or treat gastrointestinal diseases will be extensively described.

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Lactobacillus Molecular Biology: From Genomics to Probiotics | Book

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Lactobacillus is a genus of Gram-positive facultative anaerobic or microaerophilic bacteria. In humans they are symbiotic and are found in the gut flora. *Lactobacillus* species are used for the production of yogurt, cheese, sauerkraut, pickles, beer, wine, cider, kimchi, chocolate and other fermented foods, as well as animal feeds such as silage. In recent years much interest has been shown in the use of lactobacilli as probiotic organisms and their potential for disease prevention in humans and animals.

This major new work focuses on recent research on the molecular biology and genomics of *Lactobacillus*. Written by an international team of scientists the volume is an essential reference for all medical researchers, dairy technologists, microbiologists and biotechnologists in the academic and industrial sectors. Topics covered include phylogenetics, taxonomy, comparative genomics, functional genomics, the intestinal microflora, surface proteins, stress responses, interaction with the immune system, probiotics, anti-cancer potential, and much more. Essential reading for all scientists involved with lactic acid bacteria or probiotic research and a recommended book for all microbiology laboratories.

Reviews:

"... an interesting blend of fundamental and applied topics relevant to the use of these important organisms in research and industry. Fundamental aspects covered in the book are taxonomy, metabolism, stress response, genomics, and surface proteins of lactobacilli. Also included are chapters on applications of Lactobacillus strains and their potential as probiotics in the treatment of diseases such as cancer or urinary tract infections." *from* Biotechnol. J. (2009)

"... contributions from respected international scientists, many of which are leaders in their respective fields, this book constitutes an authoritative resource about both fundamental research and applications of lactobacilli. ... essential and up to date information for anyone interested in the biology of lactobacilli. The book will clearly be of interest to microbiologists, nutritionists, food scientists, and medical practitioners alike, and it is a valuable contribution to the probiotic literature." *from* Biotechnol. J. (2009)

"*Lactobacillus Molecular Biology. From Genomics to Proteomics* is an essential reference for medical researchers, microbiologists and biotechnologists, especially, dairy technologists." *from* Mercedes Berlanga, University of Barcelona writing in Int. Microbiol. (2008) 11: 294-295.

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1. Effects of *Saccharomyces boulardii* on gastrointestinal diseases: Overview in clinical trials

Several clinical trials have been performed to assess the efficacy of *S. boulardii* in the prevention and the treatment of gastrointestinal diseases which induce diarrhoea (Kotowska et al., 2005; McFarland, 2006; McFarland et al., 1994; Szajewska et al., 2007). Diarrhoea is defined as frequent watery stools resulting from impaired fluid and electrolyte absorption in the intestinal lumen, and is usually caused by pathogenic micro-organisms (Roy et al., 1995). These studies revealed the anti-diarrhoeal effects of the yeast which reduce the duration and the frequency of diarrhoea after oral administration.

Antibiotic-associated diarrhoea

Antibiotic-associated diarrhoea (AAD) is an acute inflammation of the intestinal mucosa caused by the administration of antibiotics and resulting in the disruption of the intestinal microflora. *Clostridium difficile* infection caused 10-20% of AAD and 95% of pseudomembranous colitis (Katz et al., 1996; Kelly et al., 1994). Other infectious organisms causing AAD include *Clostridium perfringens*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Candida* species and *Salmonella* species (Hogenauer et al., 1998). The risk factors to develop AAD are multiple: Host factors, antibiotherapy and exposure to nosocomial pathogens (Ackermann et al., 2005; McFarland, 1998). Several meta-analyses were performed to evaluate the efficacy of probiotics for the prevention of AAD (Cremonini et al., 2002; D'Souza et al., 2002; McFarland, 2006; Szajewska et al., 2006). These studies concluded that *S. boulardii* is effective in preventing AAD in adults and children. *S. boulardii* preventive effect on AAD was evaluated in 269 children with otitis media and/or respiratory tract infections (Kotowska et al., 2005). Children received antibiotic treatment plus 250 mg of *S. boulardii* (experimental group, $n = 132$) or a placebo (control group, $n = 137$) orally and twice a day during the antibiotherapy. Analyses included data from 246 children and demonstrated that the prevalence of diarrhoea was lower in experimental than in placebo groups (8% *versus* 23%, relative risk: 0.3, 95% confidence interval: 0.2-0.7). Moreover, in a recent study conducted on 151 hospitalized patients, the subjects were divided in two groups of 78 and 73 patients which received respectively antibiotics plus placebo and antibiotics plus *S. boulardii* twice a day (Can et al., 2006). The diarrhoea occurrence was investigated and the presence of *Clostridium difficile* toxin A in stools was evaluated by enzyme-linked immuno sorbent assay. The results showed a decrease in diarrhoea occurrence (1.4% *versus* 9% in the placebo group, $P < 0.005$). In addition, *C. difficile* toxin A assay revealed 2 positive stools samples (2/7) from patients in the placebo group while nothing was detected in *S. boulardii*-treated group (0/7) (Can et al., 2006).

Recurrent *Clostridium difficile*-associated diseases

Treatment of *Clostridium difficile*-associated diseases with metronidazole or vancomycin is effective, but in few patients, the disease recurs after the antibiotherapy. Several clinical trials have shown the efficacy of *S. boulardii* in combination with antibiotics for treating relapses of diarrhoea and colitis (McFarland, 2006; McFarland et al., 1994; Surawicz et al., 2000). In a first randomized placebo-controlled study, 124 patients treated with antibiotics plus *S. boulardii* (500 mg twice daily) had a reduction in occurrence of diarrhoea or colitis (34.6% versus 64.7% relapses in the placebo group, $P = 0.04$) (McFarland et al., 1994). Another study conducted in 170 patients showed the efficacy of *S. boulardii* (1g/d for 28 days) plus vancomycin (2g/d for 10 days) in the decrease of recurrence (16.7% *versus* 50% in the placebo group, $P = 0.05$) (Surawicz et al., 2000). This result was significant only in patients treated with high-dose of vancomycin (2g/d) and not with lower dose (500mg/d) or metronidazole (1g/d). A recent meta-analysis (McFarland, 2006) compared the efficacy of probiotics (*S. boulardii*, *Lactobacillus rhamnosus* GG and probiotics mixtures) in the prevention of AAD and the treatment of *Clostridium difficile*-diseases. The authors concluded that the three types of probiotics significantly reduced the development of AAD but only *S. boulardii* was shown to be effective for *Clostridium difficile*-associated diseases.

Traveller's diarrhoea

Traveller's diarrhoea is the most frequent disorder encountered by persons travelling from low risk regions to developing areas where enteric infection is hyperendemic. Enterotoxigenic *E. coli*, *Shigella* and *Salmonella* account for about 80% of acute traveller's diarrhoea with an identified pathogen (Sanders and Tribble, 2001). In a placebo controlled double-bind study, *S. boulardii* preventive effect on traveller's diarrhoea was evaluated on a total of 1016 travellers assigned in 3 groups (Kollaritsch et al., 1993). The first group received a placebo and the second and the third groups received *S. boulardii* (250 and 1000 mg/d, respectively). Treatment with the yeast has been assumed 5 days before the travel and during the entire trip. The incidence of diarrhoea was 39.1% in the placebo group, 34.4% in the second group ($P = 0.019$ *versus* placebo), and 28.7% in the third group ($P = 0.005$). Recently, a meta-analysis was performed to assess the efficacy of probiotics in the prevention of traveller's diarrhoea and concluded that *S. boulardii* and a mixture of *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* had a significant efficacy (McFarland, 2007).

Acute diarrhoea in children

Diarrhoeal diseases are a leading cause of childhood morbidity and mortality in developing countries. The aetiology of acute diarrhoea in children includes infections of the gastrointestinal tract due to bacteria, viruses or protozoa, intoxications, systemic infections, malabsorption disorders, nutritional deficiency, allergy and intolerance to food or drugs (Guandalini, 2000). There are several randomized placebo-controlled studies showing the efficacy of *S. boulardii* in the management and prevention of acute childhood diarrhoea (Htwe et al., 2008; Szajewska et al., 2007). A meta-analysis based on 5 randomized-controlled trials (619 participants) displayed this effect (Szajewska et al., 2007). Combined data showed that *S. boulardii* reduces significantly diarrhoea duration and the risk of prolonged diarrhoea compared with control. A recent study conducted in 100 hospitalized children showed that *S. boulardii* treatment for 5 days reduces the mean duration of diarrhoea (3.08 *versus* 4.68 days in placebo group, $P < 0.05$), the frequency of stools (on day 2: 54% *versus* 30% in placebo group had less than 3 stools/d, $P = 0.019$) and

normalizes stool consistency (on day 3: 76% *versus* 24% in placebo group, $P = 0.019$) (Htwe et al., 2008).

Diarrhoea in patients with total enteral feeding

Diarrhoea is the most frequent complication in enteral tube feeding (Whelan et al., 2001). Alterations in the colonic microflora have been identified in patients receiving enteral tube feeding and these changes may be associated with the diarrhoea incidence (Schneider et al., 2000). *S. boulardii* can be used to prevent these negative alterations and to reduce the diarrhoea incidence. The preventive effect of *S. boulardii* has been assessed in a multicenter, randomized, double-bind placebo-controlled study (Bleichner et al., 1997). A total of 128 critically ill patients needing enteral nutrition for at least 6 days were included in the study. *S. boulardii* administrated at the dose of 2 g/d reduced the mean percentage of days with diarrhoea (14.2% *versus* 18.9% in the placebo group, odds ratio = 0.67, 95%, confidence interval: 0.50-0.90, $P = 0.0069$). This study confirmed the results obtained in two previous clinical trials where *S. boulardii* administration decreased the length of diarrhoea events (Schlotterer et al., 1987; Tempe et al., 1983).

AIDS-associated diarrhoea

Acquired immune deficiency syndrome (AIDS) is a viral infection characterized by immune cell dysfunction and subsequent immunodeficiency, as well as intestinal disorder (Kotler et al., 1984). A randomized, double-bind placebo-controlled study conducted in 35 patients showed that administration of *S. boulardii* (3 g/d) for one week decreases the diarrhoea incidence. After 7 days of treatment, 61% of patients were diarrhoea-free compared with 12% in the placebo group ($P < 0.002$) (Saint-Marc et al., 1991).

Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) are characterized by a chronic course in which phases of remission of variable length are interrupted by acute episodes. The two main subforms are ulcerative colitis and Crohn's disease. Intestinal inflammation in ulcerative colitis is primarily limited to the colon whereas the whole gastrointestinal tract can be involved in Crohn's disease (Xavier and Podolsky, 2007). In IBD, genetically defects result in abnormal mucosal immune response overacting the microflora and inducing intestinal inflammation (Strober et al., 2007). In a pilot study conducted in 20 patients, administration of *S. boulardii* in addition of conventional therapy was found superior to placebo in promoting reduction of bowel movements (Plein and Hotz, 1993). Furthermore, in another clinical trial conducted in 32 patients, the preventive effect of *S. boulardii* was evaluated in relapses of Crohn's disease (Guslandi et al., 2000). After six months, patients treated with mesalamine alone (3 g/d) presented more clinical relapses compared to patients receiving *S. boulardii* (1 g/d) plus mesalamine (2 g/d) (37.5% *versus* 6.25%, $P = 0.04$). The same author evaluated the effectiveness of *S. boulardii* in the treatment of ulcerative colitis in 25 patients. Administration of *S. boulardii* (750 mg/d) plus mesalamine for 4 weeks resulted in clinical remission for 68% of patients (Guslandi et al., 2003).

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a chronic disorder involving a combination of abdominal pain and change in bowel habit and bloating. Recent evidence suggests a role of the microflora in IBS pathogenesis (Parkes et al., 2008). In a double-blind, placebo-controlled study conducted in 34 patients with predominant episodes of diarrhoea, treatment with *S. boulardii* decreased the daily number of stools ($P < 0.05$) and improved their consistency ($P < 0.05$) (Maupas et al., 1983).

Safety of administration

S. boulardii is administrated to patients in a lyophilized form and the treatment is well tolerated. However, some rare cases of *S. boulardii* fungemias have been reported in patients with an indwelling central venous catheter (de Llanos et al., 2006; Hennequin et al., 2000; Lherm et al., 2002). The origin of the fungemia is thought to be either a digestive tract translocation or a contamination of the central venous line by the colonized hands of health workers (Hennequin et al., 2000). This raises the question of the risk-benefit ratio of *S. boulardii* in critically ill or immunocompromised patients. Thus, administration of *S. boulardii* should be contraindicated for patients of fragile health, as well as for patients with central venous catheter (Herbrecht and Nivoix, 2005).

2. Experimental effects of *Saccharomyces boulardii*

These effects have been assessed in several studies and showed that *S. boulardii* exerts beneficial mechanisms in animal models displaying IBD as well as in pathogenic or opportunistic micro-organism infection models such as *Clostridium difficile*, *Vibrio cholerae*, *Escherichia coli*, *Salmonella enterica* subspecies *enterica serovar* Typhimurium, *Shigella flexneri*, *Citrobacter rodentium* and *Candida albicans* (Chen et al., 2006; Czerucka et al., 1994; Dahan et al., 2003; Dalmasso et al., 2006b; Jawhara and Poulain, 2007; Mumy et al., 2007; Wu et al., 2008). These mechanisms include the modification of host cell signalling pathways implicated in proinflammatory response and in hydroelectrolytic secretion, the stimulation of host immune defences, the neutralization of bacterial toxins and the decrease of bacterial adherence to intestinal epithelial cells, the maintenance of membrane permeability and the inhibition of pathogen translocation. Experimental effects of *S. boulardii* on diarrhoeal pathogens have been previously reviewed (Czerucka and Rampal, 2002).

2.1. Anti-inflammatory effects

Several experimental studies showed that *S. boulardii* interferes with the host cell signalling pathways and decreases the expression of inflammation-associated cytokines such as interleukin 8 (IL-8), IL-6, IL-1β, tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) (Dahan et al., 2003; Dalmasso et al., 2006b; Mumy et al., 2007). These studies showed that the yeast can reduce inflammation in blocking nuclear factor-kappa B (NF-κB) (Dahan et al., 2003; Mumy et al., 2007) and mitogen-activated protein kinase (MAPK) activation (Chen et al., 2006; Mumy et al., 2007), in decreasing nitric oxide (NO) production (Girard et al., 2005), in enhancing peroxisome proliferators-activated receptor-gamma (PPAR-γ) expression (Lee et al., 2005) and in moduling T cell migratory behaviour (Dalmasso et al., 2006a).

Inhibition of NF-κB and MAPK activation

During the course of infection, activation of NF-κB and MAPK in intestinal epithelial cells results in pro-inflammatory cytokines secretion and intestinal inflammation. *Clostridium difficile*-toxin A induces intestinal inflammation, fluid secretion and mucosal injury (Torres et al., 1990; Triadafilopoulos et al., 1987; Triadafilopoulos et al., 1989). After stimulation with toxin A, *S. boulardii* supernatant (<10 kDa) has shown to decrease IL-8 secretion in human colonocytes and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) activation in both human colonocytes and murine ileal loops (Chen et al., 2006). In a model of T84 human colonic cell line, *S. boulardii* inhibited also the NF-κB and MAPK signalling pathways in enterohemorrhagic *E. coli* (EHEC) infected-T84 cells resulting in the decrease of IL-8 and TNF-α secretion by epithelial cells (Dahan et al., 2003; Dalmasso et al., 2006b). Furthermore, the decrease of IL-8 secretion was observed in *Shigella flexneri* infected-T84 cells when the yeast or the yeast conditioned medium were added to the monolayer culture (Mumy et al., 2007). This decrease was greater for cells treated with the conditioned medium. Furthermore, polymorphonuclear leukocytes (PMN) transepithelial migration is reduced in presence of the yeast. This result could be explained by the decrease in IL-8 secretion level since this cytokine has been previously shown to be chemoattractant for PMN. *S. boulardii* decreased also phosphorilation and activation of ERK and JNK MAP Kinases and decreased the IκB phosphorilation and degradation during the infection, thereby preventing NF-κB activation (Mumy et al., 2007). These events are likely responsible for the decrease in IL-8 secretion and PMN transmigration. These anti-inflammatory effects were confirmed *in vivo* with a model of human fetal colonic tissue transplanted into severe combined immunodeficiency (SCID) mice (Mumy et al., 2007). In addition, recent data showed that the yeast produces a low molecular weight soluble factor (< 1 kDa) blocking NF-κB activation and NF-κB-mediated Il-8 gene expression in intestinal epithelial cells and monocytes (Sougioultzis et al., 2006) (Figure 1).

Decrease of nitric oxide production

In IBD, production of high levels of NO is associated with inflammatory effects (Dijkstra et al., 1998). NO is released through conversion of L-arginine to NO and L-citrulline. This reaction is catalyzed by three isoforms of the nitric oxide synthase (NOS): Neuronal NOS, endothelial NOS and inducible NOS (Moncada et al., 1991). The iNOS activity is up-regulated during immune activation and can result in the synthesis of high levels of NO. *S. boulardii* inhibitory effect in iNOS activity was displayed in the rat castor oil-induced diarrhoea model (Girard et al., 2005). The citrulline level (a marker of NO production) was increased in the colon of rats whereas *S. boulardii* administration was shown to block this production (Figure 1). The same effect is reported with the iNOS inhibitor. These results suggest that iNOS inhibition by *S. boulardii* may be beneficial in the treatment of diarrhoea and/or IBD associated with overproduction of NO (Girard et al., 2005).

Enhancement of PPAR-γ expression

PPAR-γ is a nuclear receptor expressed by several cell types including intestinal epithelial cells, dendritic cells (DC), T and B cells, and can act as a regulator of the inflammation (Escher et al., 2001; Mansen et al., 1996; Su et al., 1999). It has been shown that *S. boulardii* up-regulates PPAR-γ expression in human colonocytes at both mRNA and protein levels (Lee et al., 2005). This up-regulation is correlated with a reduction of the human colon cell response to proinflammatory cytokines (Lee et al., 2005) (Figure 1). This anti-inflammatory effect was supported by previous data showing that PPAR-γ ligands inhibited NF-κB activation and that TLR4-activated PPAR-γ in epithelium attenuated NF-κB signalling (Dubuquoy et al., 2003; Genolet et al., 2004). A recent study showed that PPAR-γ expression in colonic epithelial cells decreased pro-inflammatory cytokines levels and protects mice against experimental IBD (Adachi et al., 2006). Thus, *S. boulardii* up-regulates PPAR-γ expression which could be a therapeutic target in IBD.

Modulation of T cell migratory behaviour

In a model of lymphocyte-transferred SCID mice displaying IBD, *S. boulardii* administration decreased the production of IFN-γ and inflammation in the colon (Dalmasso et al., 2006a). This observation was correlated with a modification of T cells distribution. Indeed, the number of IFNγ-producing CD4+ T cells decreased within the colon and there were more cells in the mesenteric lymph node. *S. boulardii* appeared to be involved in the modification of lymph node endothelial cell adhesiveness and in the recruitment of IFNγ-producing CD4+ T cells to this location. These result suggested that *S. boulardii* administration could have indirectly beneficial effect in the treatment of IBD (Dalmasso et al., 2006a).

2.2. Immuno-modulatory effects

Among the mechanisms used by probiotics to improve the host health, one of them is the stimulation of the host immunity. Several studies demonstrated that *S. boulardii* might induce a protective effect in modulating both innate and adaptive host immunity to respond against pathogen infection.

Saccharomyces boulardii effect on innate immunity

S. boulardii immunopharmacological effects in healthy human volunteers have been evaluated and it has been shown that the yeast triggered the activation of the complement and the reticuloendothelial systems (Caetano et al., 1986). *In vitro*, the yeast activates the complement and the migration of monocyte and granulocyte (Caetano et al., 1986). Furthermore, *S. boulardii* administration to germ-free mice has shown to enhance the number of Küpffer cells (Rodrigues et al., 2000). These results indicate that *S. boulardii* is able to stimulate the innate immune system.

Saccharomyces boulardii effect on adaptive immunity

S. boulardii oral administration to rats and mice has shown to stimulate the secretion of immune factors (Buts et al., 1990; Qamar et al., 2001; Rodrigues et al., 2000). In the duodenal fluid of rats treated with the yeast, secretory immunoglobulin A (sIgA) mean concentration was increased by 56.9% (*versus* control, $P < 0.01$) (Buts et al., 1990). Additionally, the secretory component was significantly increased in the duodenal fluid (62.8% *versus* control, $P < 0.01$) as well as in *villus* cells (69% *versus* control, $P < 0.025$) and in crypt cells (80% *versus* control, $P < 0.01$) (Buts et al., 1990). The same effect was reported in mice challenged with *C. difficile* toxin A (Qamar et al., 2001). Treatment with *S. boulardii*

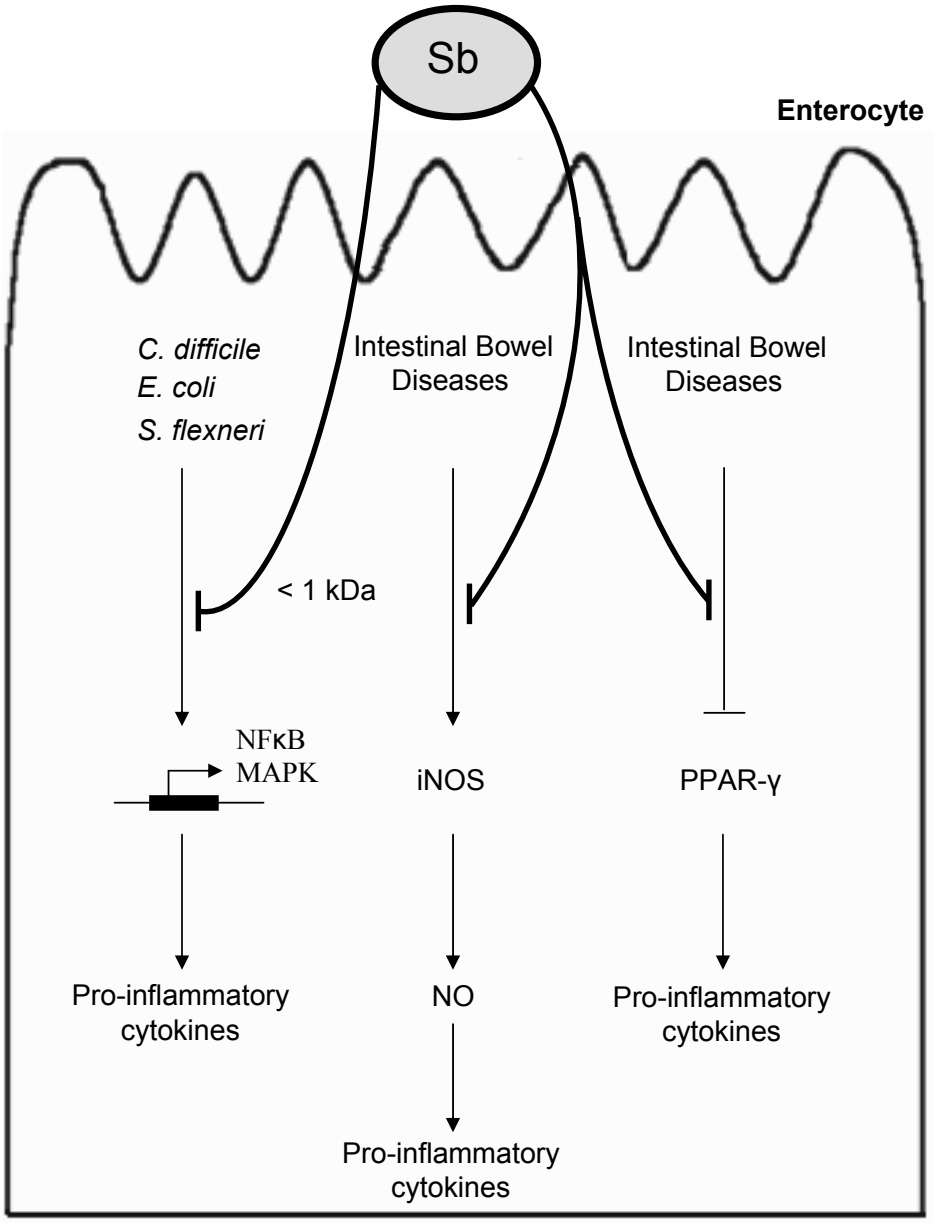


Figure 1. During the course of infection with enteric pathogens such as *Clostridium difficile*, *Escherichia coli* or *Shigella flexneri*, activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) in enterocyte leads to the secretion of inflammation associated cytokines such as IL-8 and TNF-α. *Saccharomyces boulardii* (Sb) secretes soluble factors which reduce NF-κB and MAPK activation resulting in a decrease of the inflammation. The yeast produces a low molecular weight soluble factor (< 1 kDa) blocking NF-κB-mediated IL-8 expression. In inflammatory bowel disease (IBD), production of high levels of nitric oxide (NO) is correlated with intestinal inflammation. *Saccharomyces boulardii* decreases the inducible nitric oxide synthase (iNOS) activity and consequently reduces intestinal inflammation. In IBD, inhibition of peroxisome proliferators-activated receptor-gamma (PPAR-γ), a regulator of inflammation, leads to intestinal inflammation. *Saccharomyces boulardii* up-regulates PPAR-γ expression and consequently decreases intestinal inflammation.

caused a 1.8-fold increase in total sIgA levels (P = 0.003) and a 4.4-fold increase in specific anti-toxin A sIgA levels (P < 0.001). The effect of *S. boulardii* on the immune system was also evaluated by comparison of germ-free mice mono-associated with the probiotic and germ-free mice non-associated (Rodrigues et al., 2000). Higher levels of sIgA both total and anti-*S. boulardii* were produced in the small intestine of mono-associated mice (Rodrigues et al., 2000). Thus, these results demonstrated that the yeast is able to enhance the secretion of mucosal immune factors which protect the host against enteric pathogens (Figure 2A).

After intravenous administration of *E. coli* B41, clearance of the human pathogenic strain from the bloodstream of *S. boulardii* monoassociated mice was more effective than in germ-free mice. More efficient clearance was correlated with earlier production of IFN-γ and IL-12 which are involved in the T-helper 1 response (Rodrigues et al., 2000). A further study in healthy human volunteers investigated the effect of *S. boulardii* on lymphocyte phenotype (Jahn et al., 1996). Results were compared before and after the yeast treatment. No phenotypic changes were observed in intestinal lymphocytes. However, CD4+ T cells of the peripheral blood had a significant increased expression of CD25 ($P < 0.02$) (Jahn et al., 1996). Since it has been shown that CD4+ CD25+ T cells can display regulatory functions (Sakaguchi et al., 1995; Sakaguchi et al., 2007), further studies should be performed to assess the effect of *S. boulardii* in the stimulation of regulatory T cell population and to investigate a possible link between the induction of a regulatory immune response and the decrease of inflammation.

2.3. Inhibition of hydroelectrolytic secretions

S. boulardii inhibition of hydroelectrolytic secretions was widely demonstrated in *Vibrio cholerae* infectious model (Czerucka et al., 1989; Czerucka et al., 1994; Vidon et al., 1986). *Vibrio cholerae* produces a 84 kDa toxin (CT) composed of two subunits: A and B. CT binds its intestinal receptor (monosialoganglioside GM1) through the B subunit and catalyzes the activation of adenylate cyclase through the A subunit. This activation results in an increase on cyclic adenosine monophosphate (cAMP) levels triggering active secretion of chloride and bicarbonate in crypt cells and inhibiting chloride absorption in the *villi*. This perturbation in hydroelectrolytic secretions induces severe diarrhoea (Kaper et al., 1994).

In a previous study, the model of jejunal intestinal loops in rats allowed to demonstrate the protective effect of *S. boulardii* in CT-induced pathogenesis (Vidon et al., 1986). The loops injected with CT and then with the yeast presented a significant decrease in the hydrosaline secretion. The

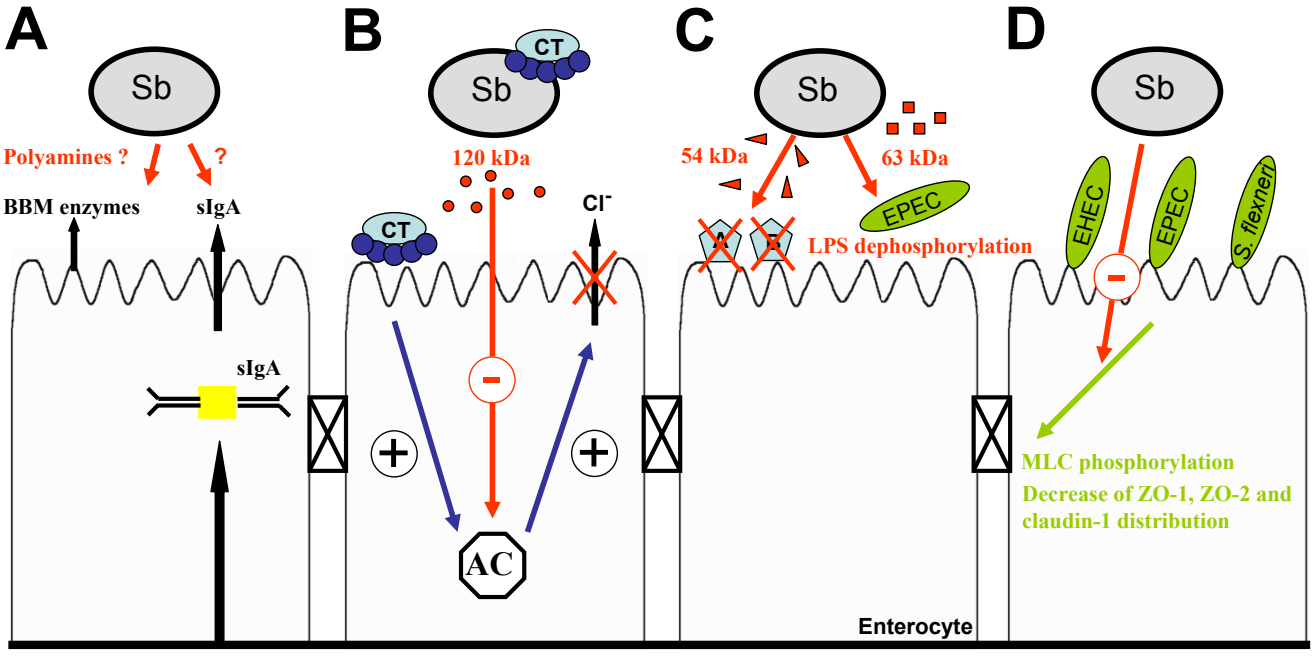


Figure 2. A: Oral administration of *Saccharomyces boulardii* (Sb) enhances both secretory IgA (sIgA) concentration in the intestinal lumen and brush border membrane enzyme (BBM) expression. The increase in their expression seems to be correlated with a secretion of polyamines during the yeast catabolism.
B: Cholera toxin (CT) binding on the intestinal epithelial cell surface induces adenylate cyclase (AC) expression resulting in chloride (Cl) secretion. *Saccharomyces boulardii* produces a 120 kDa protein which inhibits AC activation resulting in a decrease of chloride secretion.
C: *Saccharomyces boulardii* neutralizes bacterial toxins. The yeast produces respectively a 54 kDa proteinase which exerts a proteolytic activity on both *Clostridium difficile* toxins A and B and a 63 kDa protein phosphatase which dephosphorylates LPS from enteropathogenic *Escherichia coli*.
D: Enteric pathogens such as enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* and *Shigella flexneri* induce an alteration of the epithelial barrier integrity in affecting the tight-junctions-associated proteins. *Saccharomyces boulardii* abolishes the myosin light chain (MLC) phosphorilation and restores *Zonula occludens* 1 and 2 (ZO-1, ZO-2) and claudin-1 distribution. Therefore, the yeast enhances the cell ability to restore intestinal permeability.

inhibitory effect of *S. boulardii* on CT was confirmed using rats' intestinal cell lines (IRD-98 and IEC-17) (Czerucka et al., 1989). Cells pre-incubated with the yeast and challenged with the CT had a 50% decrease in the activity of cAMP. However, in this study, when *S. boulardii* was killed, the inhibitory effect on cAMP disappeared. The same author showed that incubation of IEC-6 cells (rat intestinal epithelial cells) with the yeast-conditioned medium decrease the cAMP activity induced by CT. This effect disappeared when the yeast-conditioned medium was denatured by heating, by trichloroacetic acid precipitation or by trypsin hydrolysis suggesting the presence of a secreted factor which has been characterized as a 120 kDa protein (Czerucka et al., 1994) (Figure 2B). These results drove Czerucka and Rampal to evaluate in T84 cells the effect of *S. boulardii*-conditioned medium (*Sb*-CM) on chloride secretion mediated by cAMP and calcium. Preincubation of cells with *Sb*-CM reduced chloride secretion mediated by cAMP agonists (CT, prostaglandin E_2, vasoactive intestinal polypeptide and forskolin) and by charbachol, a muscarinic agonist implicated in the calcium signalling pathways (Czerucka and Rampal, 1999). These studies demonstrate that *S. boulardii* secrets a 120 kDa protein that interferes with chloride secretion pathways and decreases diarrhoea caused by *Vibrio cholerae* infection. An additional mechanism of protection was proposed with the adhesion of CT to a receptor on the yeast surface (Brandao et al., 1998). The authors suggested that the yeast receptor could be able to bind the B subunit while the A subunit was internalized and stimulated cAMP signals as well as cAMP-dependent trehalase activity. This result supposes that the yeast and the mammalian CT receptors could be structurally and functionally similar.

2.4. Neutralization of bacterial toxins

It has been shown that *S. boulardii* has the capacity to release factors which neutralize bacterial toxins and decrease the deleterious effects of infectious pathogens (Buts et al., 2006; Castagliuolo et al., 1996; Castagliuolo et al., 1999). *C. difficile* is responsible of AAD in humans and animals, and is one of the most common nosocomial pathogens. Pathogenic *C. difficile* produces two exotoxins, toxin A and toxin B which induce intestinal inflammation, fluid secretion and mucosal injury (Pothoulakis, 1996). *S. boulardii* produces a 54 kDa serine protease exerting a proteolytic activity on both toxin A and B (Castagliuolo et al., 1996; Castagliuolo et al., 1999). This proteolysis results in the inhibition of toxins binding on their intestinal receptors (Figure 2C). Thus *S. boulardii* treatment enhances transepithelial resistance, epithelial barrier integrity and decreases hydroelectrolytic secretions in both rat ileum and colonic mucosa. *S. boulardii* produces also a 63 kDa protein phosphatase that inhibits the LPS toxicity of enteropathogenic *E. coli* O55B5 by endotoxin dephosphorylation (Buts et al., 2006) (Figure 2C). Intraperitoneal injection of dephosphorylated LPS in rats decreases the level of circulating TNF-α and abolishes inflammatory lesions in comparison to rats injected with native LPS.

2.5. Decrease of bacterial adhesion to intestinal epithelial cells

C. rodentium is a micro-organism that colonizes the colon of mice, causing "attaching/effacing" lesions and colonic hyperplasia (Schauer and Falkow, 1993). In addition to serving as model for EPEC and EHEC infection in small-animal, *C. rodentium* can also serve as model for inflammatory bowel disease in mouse (Higgins et al., 1999). Recently, the beneficial effect of *S. boulardii* on *C. rodentium*-induced colitis was assessed in mice (Wu et al., 2008). The improvement effect of *S. boulardii* was associated with significantly reduced numbers of mucosal adherent bacteria compared with infected untreated animals ($P < 0.05$). This effect was not due to a bactericidal action but was correlated with a reduction in EspB and Tir protein secretions, respectively a translocator and an effector protein implicated in the type III secretion system (TTSS). The authors concluded that *S. boulardii* maintains the epithelial barrier integrity and ameliorates inflammatory lesions associated with *C. rodentium* infection by attenuating bacterial adherence to the host cells through putative actions on TTSS (Wu et al., 2008). However, in some *in vitro* models such as EPEC-infected T84 cells or *Shigella*-infected T84 cells (Czerucka 2000, Mumy 2007), *S. boulardii* did not alter the number of adherent bacteria.

2.6. Maintenance of epithelial barrier integrity

Several pathogenic microorgansims such as *Shigella flexneri*, enterohemorrhagic and enteropathogenic *E. coli* have mechanisms of infection characterized by bacterial adhesion to the intestinal mucosa resulting in alteration of tight-junctions, disruption of membrane permeability and enterocyte secretion of pro-inflammatory cytokines. *S. boulardii* acts on the epithelial barrier in improving tight-junctions structure and in restoring membrane permeability disrupted by infectious pathogens (Czerucka et al., 2000; Dahan et al., 2003; Mumy et al., 2007). In EPEC E2348/69-infected T84 cells the monolayer transepithelial resistance was decreased and the perijunctional distribution of tight-junction-associated protein *Zonula occludens* (ZO-1) was altered, resulting in the disruption of epithelial barrier structure (Czerucka et al., 2000). In contrast, when the infection was performed in the presence of *S. boulardii*, the transepithelial resistance was not altered and ZO-1 protein distribution was preserved, suggesting a protective effect of *S. boulardii* on the tight-junctions structure of T84-infected cells (Czerucka et al., 2000) (Figure 2D). Moreover, the yeast prevented the caspase-3 activation induced by EPEC infection and delayed apoptosis of T84 cells. In this study, *S. boulardii* decreases EPEC induced ERK1/2-activation which seems to be correlated with the decrease of the number of intracellular bacteria (Czerucka et al., 2000). This study demonstrates that *S. boulardii* modulates the signalling pathways induced by bacterial infection and thus exerts protective effects. The same effects were observed after pre-incubation of *S. boulardii* in EHEC-infected T84 cells (Dahan et al., 2003). During the bacterial infection, the myosin light chain protein (MLC) is phosphorilated and the tight-junctions are disrupted. The MLC is associated with a cytoskeletal protein in intercellular tight-junctions control. The yeast abolished MLC phosphorilation and allowed to preserve the barrier function (Figure 2D). In *Shigella*-infected T84 cells, the yeast positively affects tight-junctions proteins (claudin-1 and ZO-2) and significantly protects the barrier function (Mumy et al., 2007) (Figure 2D). *Shigella*-infected cellular monolayer had a dramatic decrease in claudin-1 and ZO-2 levels. In the presence of the yeast, cellular

monolayer exhibited larger amounts of these proteins. After 2 hours of infection, monolayers exposed to both bacteria and yeast displayed 75% of the barrier function compared to uninfected controls (*versus* 50% for monolayers exposed to bacteria alone). These results demonstrate that *S. boulardii* enhances the cell ability to restore tight-junctions structure and barrier permeability.

2.7. Trophic effects on intestinal mucosa

Acute and chronic gastrointestinal diseases often induce diarrhoea. The microflora and the intestinal mucosa are disrupted, resulting in a deficit of intestinal enzymatic activity and transporter expression, and an increase of inflammation and water loss. Several studies have shown that *S. boulardii* exerts trophic effects restoring the intestinal homeostasis (Buts et al., 1986; Buts et al., 1999; Buts et al., 2002; Jahn et al., 1996; Schneider et al., 2005).

A study conducted in human volunteers and in growing rats showed that oral administration of *S. boulardii* for 8 days increased the activity of sucrase-isomaltase (+82%), lactase (+77%) and maltase-glucoamylase (+75%). This increase was also observed in growing rats. However, this treatment did not modify the intestinal morphology (Buts et al., 1986). These results were confirmed in another study reporting an increase in lactase, α-glucosidase and alkaline phosphatase activities after the yeast treatment (Jahn et al., 1996). These results showed that *S. boulardii* enhances the release of brush-border membrane enzymes implicated in the nutrient degradation and absorption. These intestinal enzymes are often altered during acute or chronic enteropathies. In a further study, oral administration of the yeast during 8 days after a 60% proximal enterectomy in rats improved dissacharidase activities, increased the absorption of D-glucose coupled to Na^+ as well as the expression of the sodium/glucose cotransporter-1 (SGLT-1) (Buts et al., 1999). SGLT-1 is implicated in water and electrolytes reabsorbtion. Thus, these trophic effects enhanced by *S. boulardii* could be efficient in the treatment of diarrhoea and congenital sucrase-isomaltase deficiency. Buts and colleagues suggested that the increased expression of intestinal enzymes could be in part due to an endoluminal release of polyamines by *S. boulardii*, mainly spermine and spermidin (Buts et al., 1994) (Figure 2A). When rats were treated with 100 mg of lyophilized *S. boulardii* containing 679 nmol of polyamines or with 500 nmol of purified spermine, similar enzymatic responses were observed with an increase in sucrase and maltase activities. Another effect of the yeast is based on the modification of luminal short-chain fatty acids (SCFAs) concentration (Schneider et al., 2005). SCFAs which are among the most important metabolites produced by anaerobic bacteria in the colon are involved in water and electrolyte absorption by the colonic mucosa (Bowling et al., 1993). Patients on long-term total enteral nutrition have a decrease in the number of fecal anaerobic bacteria and in fecal SCFAs concentration (Schneider et al., 2000). A study showed that TEN-patients treated with *S. boulardii* had an increase in total fecal SCFAs levels and total SCFAs remained high 9 days after discontinuation of the treatment (Schneider et al., 2005). The author concluded that this increase may explain the preventive effects of the yeast in TEN-induced diarrhoea. Finally, a further study demonstrated that the yeast releases a leucine aminopeptidase enhancing endoluminal N-terminal peptide hydrolysis in suckling rat small intestine and potentially preventing reactions to food antigens when the barrier permeability is altered (Buts et al., 2002).

Conclusion

Gastrointestinal diseases are characterized by an alteration of the microbial balance and the intestinal homeostasis. Several clinical trials and experimental studies displayed the role of *S. boulardii* as a good biotherapeutic agent allowing to prevent and/or treat several grastrointestinal diseases. In comparison to probiotic bacteria, the use of probiotic yeast is beneficial when the treatment is combined to antibiotherapy. Oral treatment with *S. boulardii* induces modulation of the host cell signalling pathways implicated in proinflammatory response and in hydroelectrolytic secretion, neutralization of bacterial toxins, inhibition of pathogen translocation, stimulation of the host immune response, restoration of intestinal permeability, and stimulation of brush-border membrane enzymes and transporters. Thus, *S. boulardii* is involved in the restoration of intestinal homeostasis. These beneficial effects are in part mediated by secreted factors such as proteases, phosphatases and polyamines.

The recent advance in mucosal immunology understanding and the discovery of *S. boulardii* immuno-modulatory and anti-inflammatory effects open a new field of perspectives. Several reports showed that *S. boulardii* induces a local mucosal protection in the gut (Buts et al., 1990; Qamar et al., 2001; Rodrigues et al., 2000). However, further studies should be performed to evaluate if the yeast can proceed as an “immunobiotic” by modulating adaptive immune factors to act in other mucosal sites than the gut as the bronchial or the uro-genital mucosa (Clancy, 2003). Another question is to identify if the yeast can activate adaptive immune factors involved in the entero-mammary link, conferring better protection to the newborn (Bourges et al., 2008). Indeed, it has been shown that the yeast can modulate the migratory behaviour of lymphocytes by modification of lymph node endothelial cell adhesiveness (Dalmasso et al., 2006a). Then, a better characterization of the pathways involved in the anti-inflammatory response and the molecular mechanisms implied is required. The discovery of T regulatory cells with anti-inflammatory functions could lead to investigate if *S. boulardii* is able to stimulate this population. In addition, *S. boulardii* effect on DCs could be investigated to identify if the yeast could stimulate DC regulatory functions by targeting specific pattern-recognition receptors.

In conclusion, *S. boulardii* activities in the prevention and/or the treatment of diarrhoea have been widely investigated and demonstrated. However, new data and further experimental studies should permit to better elucidate the mechanisms of action of the yeast and suggest new therapeutic applications.

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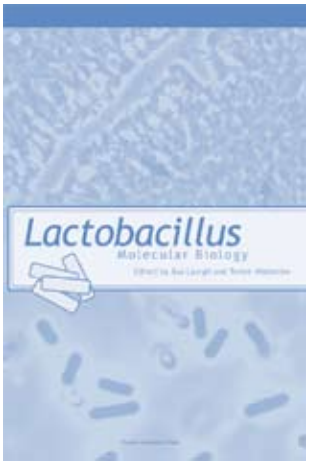
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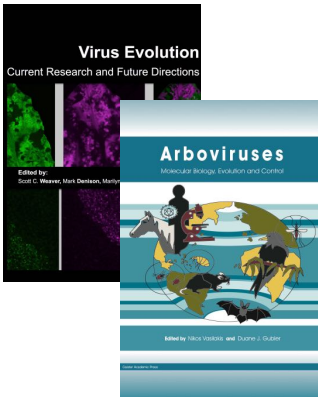
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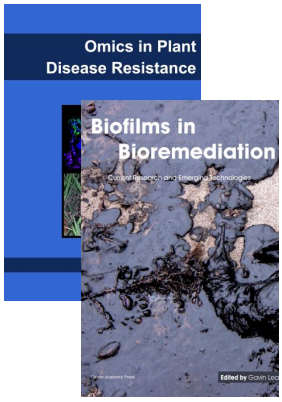
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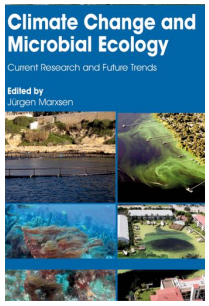
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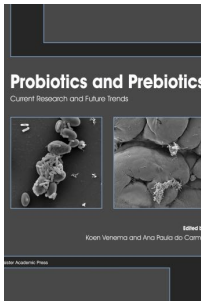
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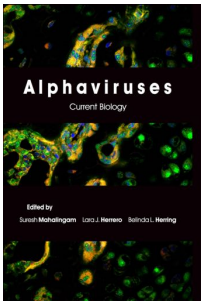
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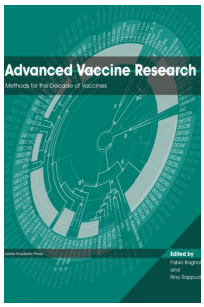


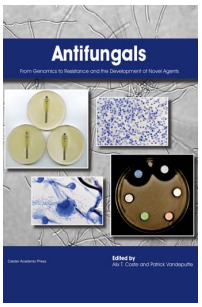












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