

Probiotics for the treatment of *Clostridium difficile* associated disease

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Abstract

The purpose of this review paper is to update the current and potential future role of probiotics for *Clostridium difficile*-associated disease (CDAD). Included in this review, is an update on the testing of newer probiotics (*e.g.*, *Bacillus coagulans* GBI-30, 6086) in animal models of CDAD. There is a focus on the modulation of signal transduction pathways (*i.e.*, transcription factors like cAMP response element-binding, activator protein 1, and nuclear factor kappa B), as well as the inhibition of certain kinases (*e.g.*, p38 mitogen activated protein kinases) by probiotics. Inhibition of signal transduction by probiotics, such as *Saccharomyces boulardii*, result in multiple effects on intestinal fluid secretion, neutrophil influx into the colon, inflammation, and colonocyte apoptosis that may positively impact CDAD. Recent clinical approaches with probiotics, for the prevention of primary and recurrent CDAD, are also summarized in this review paper. Future directions for the treatment of CDAD by probiotics are also mentioned in this review. In particular, the use of multi-strain probiotic formulations such as Ecologic[®] AAD and VSL #3[®] may represent a rationale pharmacological approach, particularly as adjunctive therapies for CDAD. Understanding the mechanistic basis of CDAD, and how probiotics interfere at certain steps in the pathogenic process, may also present the opportunity to design other multi-strain

probiotics that could have a future impact on CDAD.

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Key words: *Clostridium difficile*; Colitis; Probiotics; Mechanisms of action; Immune modulation; Transcription factors; *Saccharomyces boulardii*; VSL#3

Core tip: Certain probiotics can inhibit signal transduction pathways (*i.e.*, transcription factors like cAMP response element-binding, activator protein 1, and nuclear factor kappa B), as well as attenuate the activation of certain kinases (*e.g.*, p38 mitogen activated protein kinases). Inhibition of these intracellular signaling pathways by probiotics results in effects on intestinal fluid secretion, neutrophil influx into the colon, inflammation and colonocyte apoptosis that may positively impact *Clostridium difficile*-associated disease (CDAD). Understanding the mechanistic basis of CDAD, and how probiotics interfere at certain steps in the pathogenic process, may allow the development of novel probiotics that could have a future pharmacological impact on CDAD.

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INTRODUCTION

Clostridium difficile (*C. difficile*) infection can cause nosocomial-related diarrhea and other distinct disease characteristics, which can affect the structural integrity of the intestine^[1,2]. The spectrum of *C. difficile*-associated disease (CDAD) ranges from mild antibiotic associated diarrhea to severe pseudomembranous colitis that can lead

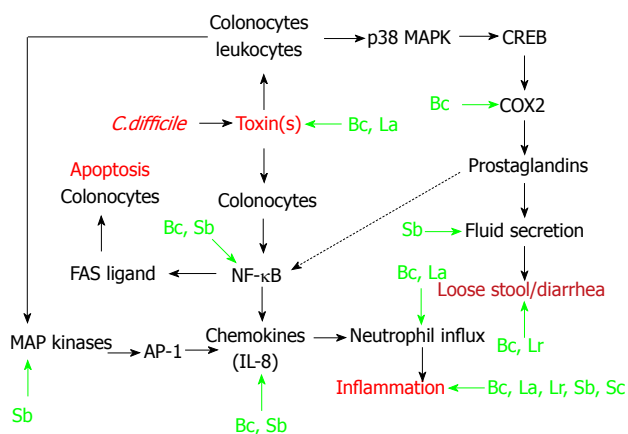


Figure 1 Immunomodulation by probiotics for *Clostridium difficile*-associated disease. *Clostridium difficile* (*C. difficile*) associated toxins (red font) engage colonic epithelial cells (colonocytes) leading to nuclear factor-kappa B (NF-κB) activation, interleukin (IL)-8 production, neutrophil influx and inflammation. These toxins also bind to receptors on colonocytes and leukocytes leading to p38 mitogen activated protein kinases (p38 MAPK) and cyclic-AMP response binding protein (CREB) activation. CREB, through cyclooxygenase 2 (COX2), is critical for the production of prostaglandin E2. In turn, this prostaglandin plays an important role in the fluid secretion/diarrhea associated with CDAD. *C. difficile* associated toxins also lead to the activation of other MAP kinases (ERK 1/2) and activator protein-1 (AP-1), which also plays a role in IL-8 production. There is also cross talk (dotted line) between the various pathways. For example, prostaglandin E2 can stimulate Fas ligand expression and apoptosis in colonic epithelial cells. The green arrows in this figure represent specific points of intervention by certain probiotics, resulting in immunomodulation by these agents. The abbreviations indicate the specific probiotics, which can modulate these signal transduction pathways. These probiotics (green font) include: *Saccharomyces boulardii* (Sb); *Bacillus coagulans* GBI-30, 6086 (Bc); *Lactobacillus acidophilus* (La); *Lactobacillus rhamnosus* (Lr); and *Saccharomyces cerevisiae*, strain 905 (Sc).

to mortality^[1,2]. CDAD is caused by the actions of two exotoxins (toxin A and toxin B), which are produced by various pathogenic strains of *C. difficile*^[2,3].

CDAD is often treated successfully with standard antibiotics such as vancomycin and metronidazole^[4-6]. However, recurrence occurs in many patients^[4-6]. Some clinical studies have focused on combined treatment with vancomycin and probiotics such as *Saccharomyces boulardii* for the treatment of recurrence^[7-10]. Therefore, initial treatment regimens with probiotics, or their use for prevention of recurrent disease, may be attractive as part of the overall therapeutic strategy for CDAD^[11-13].

Probiotics are live microorganisms that when ingested can confer health benefits^[14]. Typically, probiotics include various strains of *Lactobacillus* and/or *Bifidobacteria* species. They exist as either single entities, or as combination products (e.g., VSL#3)^[15,16]. Other known probiotics include certain non-pathogenic *Escherichia coli* strains like Nissle 1917 and M-17^[14,17].

Overall, the pertinent mechanisms explaining the potential role of probiotics as anti-colitis therapies have been reviewed in detail elsewhere^[15,18-20]. The purpose of this review paper is to provide an update on the current and potential future role of probiotics for CDAD. Included in this review will be an update on the recent

testing of some probiotics in animal models of CDAD, as well as how certain probiotics can modulate signal transduction pathways.

MECHANISMS OF ACTION FOR PROBIOTICS

Focus on modulation of signal transduction (immunomodulation)

In an excellent review paper, Hell *et al*^[19] cited potential mechanisms by which probiotics could prevent or reverse CDAD. These mechanisms included: (1) competitive exclusion; (2) bacterial metabolic activity; (3) preservation of gut-barrier function; (4) influence on water and ion channels; (5) influence on the innate nervous system; (6) modulation of signal transduction; (7) stimulation of the innate immune system; and (8) induction of adaptive immunity^[19]. Specific details on these mechanisms are provided elsewhere in the relevant literature^[18,19].

In this review, I will focus on the modulation of signal transduction pathways (*i.e.*, immunomodulation) by probiotics, as related to CDAD^[19,21,22]. As shown in Figure 1, endogenous colonic epithelial cells (colonocytes) seem to play an integral role in CDAD^[23,25]. However, cells of the innate immune system (macrophages, neutrophils) also play a role in the etiology of CDAD^[26,27]. In these cellular populations within the intestine, *C. difficile* associated toxins (particularly toxin A) result in the activation of three transcription factors (Figure 1). Nuclear factor-kappa B (NF-κB) is involved in chemokine production, and also plays a role in colonocyte apoptosis^[23,28]. Activator protein-1 (AP-1) also plays a role in interleukin (IL)-8 production, in response to stimulation of colonocytes with toxin A^[24]. Cyclic-AMP response binding protein (CREB) is critical for the production of Prostaglandin E₂^[23]. This prostaglandin plays an important role in the fluid secretion/diarrhea associated with CDAD (Figure 1). As shown in the figure, there is also cross talk between the various pathways. For example, prostaglandin E₂ can stimulate Fas ligand expression and apoptosis in colonic epithelial cells^[28,29].

Specific points of intervention, resulting in immunomodulation by certain probiotics, are shown in Figure 1^[21,30-39]. The non-pathogenic yeast probiotic, *Saccharomyces boulardii* has the most well described immunomodulatory actions^[21]. *Saccharomyces boulardii* can inhibit toxin-A receptor binding to target cells, by release of a protease that digests both the exotoxin and its receptor binding sites^[21,31,32]. Indirectly, this prevents the downstream activation of relevant MAP kinases, as well as transcription factor activation by toxin A (Figure 1). The same group of investigators showed that *Saccharomyces boulardii* supernatants could inhibit (*in vitro* or *in vivo*) toxin A-induced MAP kinase (ERK 1/2) activation, IL-8 production, fluid secretion, and intestinal inflammation^[21,30]. *Saccharomyces boulardii* also reportedly inhibits activation of the key transcription factor NF-κB^[21].

Bacillus coagulans GBI-30, 6086 (Bc) is a novel probi-

otic, which can attenuate chemokine release both *in vitro* and *in vivo*^[34,35]. Correspondingly, this probiotic reduced neutrophil influx and colonic inflammation associated with CDAD in mice^[34,35]. Of note, *Bacillus coagulans* GBI-30 reduced the expression (by immunohistochemistry) of COX-2 in the colons of mice with CDAD (Figure 1)^[34,35].

Lactobacillus acidophilus (*L. acidophilus*) substantially improved cyclosporine-induced *C. difficile* infection in mice^[36,39]. Various parameters of infectious colitis were attenuate by probiotic treatment, including myeloperoxidase and histopathology, as well as titers of toxins A and B derived from the cecal contents of mice (Figure 1)^[36,39]. Another lactobacillus species, *Lactobacillus rhamnosus* (*L. rhamnosus*) improved *C. difficile*-induced inflammation and damage to the ileum of hamsters, with less evidence of diarrhea^[37].

Martins *et al*^[38] developed a screening paradigm for yeast probiotic strains, based upon protection against enteric pathogens including *C. difficile*. These investigators found that *Saccharomyces cerevisiae*, strain 905 protected the cecum of gnotobiotic mice from *C. difficile*-induced pathological changes in the cecum (Figure 1)^[38].

PROBIOTICS AND PRECLINICAL MODELS OF CDAD

Table 1 shows a list of some probiotics that were tested in pre-clinical models of *C. difficile*-induced colitis. Early studies, which were conducted approximately 30 years ago, showed that *Saccharomyces boulardii* could prevent Clindamycin (and by association *C. difficile*)-induced mortality in hamsters, with improvement in the histological appearance of the intestine in these animals^[40,41]. In the same time period, Corthier *et al*^[42] found that *Saccharomyces boulardii* could limit mortality in gnotobiotic mice that were infected with *C. difficile*. Of note, this probiotic also modulated fecal cytotoxin production (Figure 1)^[42].

More recent studies, showed that *Saccharomyces cerevisiae* strain 905 and two lactobacillus strains (*L. rhamnosus* and *acidophilus*) were effective against CDAD in rodents (Figure 1 and Table 1)^[36-39]. My laboratory found that the novel probiotic strain *Bacillus coagulans* GBI-30, 6086 could improve both the initial phase of colitis in mice following *C. difficile* infection, as well the recurrence of CDAD following vancomycin withdrawal^[34,35]. This probiotic most profoundly affected the stool consistency in these mice (Figure 1)^[34,35].

CLINICAL USE OF PROBIOTICS FOR CDAD

Since 2011, several comprehensive reviews have been published regarding the use of probiotics for CDAD. Specific details from these reviews can be found in the relevant literature^[19,43-46]. Floch *et al*^[43] gave probiotics a B/C recommendation for both the prevention of CDAD,

Table 1 Effects of probiotics in animal models of *Clostridium difficile*-induced colitis

Probiotic	Species	Efficacy	Reference
<i>Saccharomyces boulardii</i>	Hamster	Yes	[40,41]
<i>Saccharomyces boulardii</i>	Mice	Yes	[42]
<i>Saccharomyces cerevisiae</i> 905	Mice	Yes	[38]
<i>Lactobacillus rhamnosus</i>	Hamster	Yes	[37]
<i>Lactobacillus acidophilus</i>	Mice	Yes	[36,39]
<i>Bacillus coagulans</i> GBI-30, 6086	Mice	Yes	[34,35]

and also the prevention of recurrent CDAD. Their somewhat arbitrary rating system suggested some positive clinical studies, but also the presence of some negative studies (B rating), or inadequate clinical experience (C rating). In their evaluations, the investigators focused mainly on studies involving *Saccharomyces boulardii* and *Lactobacillus GG*^[43]. In another review, Hickson^[44] suggested that the evidence supporting the use of probiotics for CDAD is overall equivocal. Musgrave *et al*^[45] reported that probiotics could be considered for the prevention of *C. difficile* infection, or as an adjunctive therapy in otherwise healthy (non-immunocompromised) patients. Davidson *et al*^[46] suggested the possible co-administration of probiotics for prevention of CDAD in patients at increased risk for developing disease. However, they did not recommend adjunctive probiotics for the routine treatment of CDAD^[46]. The most recent Cochrane review (from 2008) on probiotics for CDAD in adults concluded that insufficient evidence existed to recommend probiotics as an adjunct to antibiotic therapy for *C. difficile* colitis^[47]. Moreover, reportedly there was no evidence to support the use of probiotics alone for *C. difficile* colitis^[47].

FUTURE USE OF PROBIOTICS FOR CDAD

In a review article published in 2009, Imhoff *et al*^[13] asked this question: Is there a future for probiotics in preventing *clostridium difficile*-associated disease and treatment of recurrent episodes? This statement remains a pertinent question in 2013. Recently, there has been a renewed interest in fecal microbiota transplantation therapy for recurrent CDAD, and new studies suggest efficacy for this indication^[48-52]. Therefore, what about the future use of probiotics in CDAD beyond 2013? Because CDAD is a condition associated with disrupted endogenous gut flora, it is logical to employ treatment strategies that can reconstitute/restore the physiological intestinal flora. In a broad sense, both probiotics and fecal microbiota transplantation therapy attempt to accomplish this restoration of physiological bacterial species, but by different administration methods^[13,53,54]. Certainly, fecal transplantation has yielded some interesting efficacy results^[48-54]. However, it typically requires an invasive procedure (*e.g.*, colonoscopy), as well as an overall technique that is still aesthetically displeasing to some patients^[48-54]. In contrast, probiotics can be easily ingested, but are often not optimally formulated to survive transit through the GI tract

for colonization in the colon^[13,19]. Moreover, probiotics have demonstrated questionable efficacy for CDAD^[43-47]. A recent publication further compares the pros and cons of probiotics versus fecal transplantation for intestinal diseases^[55].

With respect to the future of probiotics for CDAD, Hell *et al*^[19] have provided some good insights, as well as interesting initial clinical data. They postulated that a multi-strain probiotic, resembling a healthy human microbiota, would be most effective for treating CDAD^[19]. Therefore, these investigators developed a probiotic mixture (Ecologic[®] AAD) comprised of several *Bifidobacterium* and *Lactobacillus* strains, as well as *Enterococcus faecum*^[19]. In a small series of 10 patients (five with recurrent disease) excellent results were obtained in all evaluable patients with *C. difficile* infection, following combined treatment with the multi-strain probiotic plus vancomycin^[19]. This type of therapeutic paradigm seems to represent a logical future scientific approach for probiotic treatment in CDAD. Another probiotic preparation that could be tested for CDAD is VSL#3. This probiotic mixture contains 4 *Lactobacillus* strains, three strains of bifidobacteria and *Streptococcus salivarius*^[56,57]. VSL#3 has been tested previously in both IBD and pouchitis patient populations, with some evidence of efficacy^[56,57]. Moreover, the pertinent mechanism(s) of action for VSL#3 suggest that it would represent a rational pharmacological approach for CDAD^[14,58]. Finally, it may be possible to utilize known mechanism of action diagrams, like in Figure 1, to create novel probiotic mixtures that could potentially be effective for CDAD.

While perhaps focusing on multi-strain probiotics, newer single strain probiotics of potential interest for CDAD could include *Bifidobacterium animalis* AHCT^[59-61]. This probiotic can inhibit NF- κ B activation, reduce *C. difficile* levels in the canine colon, and resolve idiopathic diarrhea in dogs. The pharmacological profile of *Bifidobacterium animalis* AHCT suggests that it could be an interesting candidate for further testing related to CDAD. Another single strain probiotic of interest is *Clostridium butyricum* MIYARI 588, which is being used for the prevention of CDAD in Japan^[62].

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