Probiotic bacteria for prophylaxis and therapy of candidiasis

Edward Balish[1] and R. Doug Wagner[2]

[1]University of Wisconsin Medical School, Departments of Surgery and Medical Microbiology and Immunology, Madison, WI 53706-1532 and [2]National Center for Toxicological Research (NCTR), Microbiology Department, Jefferson, AR 72079-9502, USA

Summary

A good deal of data support a role for probiotic intestinal bacteria in the prophylaxis and therapy of candidiasis. *Candida* spp. are highly infectious eucaryotes that can colonize and infect humans and other warm-blooded mammals, worldwide. Although most humans manifest antibody- and cell-mediated immune responses to *Candida* antigens a large percentage of the human population is colonized with *Candida* spp. in their alimentary and vaginal tracts. The bacterial flora plays a very important probiotic role in the prophylaxis of candidiasis by suppressing the growth of *Candida* spp. on mucosal and cutaneous surfaces; however, the specific bacteria and the mechanisms they use to inhibit *Candida* spp. and candidiasis are still poorly understood. The increased incidence of *Candida* infections, their increasing resistance to antifungal antibiotics and the fact that vaccines to protect against candidiasis are not yet available (and may not work in immunodeficient, *Candida*-susceptible, patients) provides a strong impetus for new research efforts to explore the use of probiotic, anti-*Candida* intestinal bacteria for the prophylaxis and therapy of candidiasis.

Key words Candidiasis, *Candida* spp. probiotics, Intestinal flora, Biotherapy

Bacterias probióticas en la profilaxis y la terapia de la candidiasis

Resumen

Existe abundante información que apoya la existencia de un papel de las bacterias intestinales probióticas en la profilaxis y el tratamiento de las candidiasis. Las especies de *Candida* son eucariotas altamente infecciosos que pueden colonizar e infectar al ser humano y a otros mamíferos en todo el mundo. Aunque la mayoría de los seres humanos presentan respuestas inmunes celular y humoral frente a los antígenos de *Candida,* un porcentaje importante de la población humana presenta colonización del tracto alimentario y vaginal por especies de *Candida.* La flora bacteriana juega un importante papel probiótico en la profilaxis de la candidiasis limitando el crecimiento de *Candida* en las superficies cutaneomucosas. Sin embargo, se conocen aun poco las bacterias concretas y los mecanismos mediante los que inhiben a *Candida* y la candidiasis. El aumento en la incidencia de las candidiasis, su creciente resistencia al tratamiento antifúngico y la falta de disponibilidad, por el momento, de vacunas protectoras frente a la candidiasis (que además podrían ser inefectivas en pacientes inmunodeficientes) estimula la dedicación de esfuerzos en la investigación sobre la utilización de bacterias intestinales probióticas anti-*Candida* en la profilaxis y terapia de las candidiasis.

Palabras clave Candidiasis, Probióticos de *Candida* spp., Flora intestinal, Bioterapia

Candida spp. are a significant clinical problem for a variety of immunocompetent and immunocompromised patients, worldwide [1-6]. Candidiasis has increased substantially over the last decade and *Candida* spp. now rank fourth among microbes most frequently isolated from blood cultures [7] and they are the most common opportunistic pathogen in AIDS patients [8]. Paradoxically, modern medical practice which has prolonged the survival of a large number of patients who have either congenital or acquired (through disease or therapy) immunodeficiencies [1,2,6] has also made them more susceptible to candidiasis.

The increased incidence of candidiasis, the increasing resistance of *Candida* spp. to antifungal agents and

Dirección para correspondencia:
Dr. Edward Balish
University of Wisconsin Medical School, Departments of Surgery and Medical Microbiology/Immunology, 1300 University Avenue, 4638 MSC, Madison, WI 53706-1532, USA
Tel.: +1-608 2631670; Fax: +1-608 2653461;
E-Mail: balish@surgery.wisc.edu

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the rise in lethality associated with infections by *Candida* spp. [5-12] has prompted renewed research on innovative ways to prevent and treat infections by these opportunistic yeasts. New research initiatives are putting a great deal of emphasis on studies of virulence factors, vaccines and new antifungal agents; however, very little research is being carried out to increase our knowledge about a very important host defense mechanism against candidiasis; namely the *Candida*-inhibiting activities of commensal bacteria that reside in the alimentary and vaginal tracts of humans. Studies on probiotic bacteria should be included in renewed research efforts on candidiasis because they have great potential to provide very effective mechanisms for the prophylaxis and therapy of this serious and often fatal disease of immunodeficient patients.

Probiotic microbes are defined as microorganisms that are consumed to improve the health status of humans and animals [13]. Probiotic bacteria present in the alimentary tract and vagina prevent the overgrowth of *Candida* spp. and thereby decrease the likelihood of mucosal or systemic candidiasis [1,2,14]. The *Candida*-inhibitory bacteria present in the microbial flora of man and animals fulfill a very important ecological role in protecting the host from these pathogenic yeasts.

Probiotic microbes not only suppress the growth of *Candida* spp. in the alimentary tract and vagina, but they also inhibit the adherence of *Candida* spp. to epithelial surfaces [1,2,15,16]. The ultimate achievement that could be realized in using probiotic bacteria to prevent candidiasis would be to have them completely eliminate *Candida* spp. from the human microflora. Achieving the latter goal would eliminate or dramatically decrease the incidence of clinical candidiasis. Indeed the eradication of *Candida* spp. from the normal flora may actually take place in some humans who manifest antibody- and cell-mediated immune responses to *Candida* spp. but have no culturable *Candida* spp. in their alimentary tract or vagina [1,2].

Candida spp. have many unique properties that differentiate them from other microbes present on the mucosal surfaces of humans and warm-blooded mammals. For example, *Candida* spp. are eucaryotic whereas the majority of normal flora microbes are procaryotes. *Candida* spp. are normally not the predominant microbe in the human microbial flora [1,2]. Unlike most normal flora bacteria which fail to evoke antibody- and cell-mediated immune responses in the host [17], *Candida* spp. are able to colonize and infect most humans at an early age and induce strong antibody- and cell-mediated immune responses [1]. Early encounters with *Candida* spp. likely cause subclinical candidiasis that evokes both antibody- and T-cell-mediated immune responses in their host which (at least in experimental animals) can enhance resistance to the pathogenic yeasts (natural immunization) [14,18,19]. Whereas, certain species of normal flora bacteria appear to predominate in discrete sections of the alimentary tract, vagina, or skin, *Candida* spp., except for the unwashed hands of hospital personnel [5,9,11], do not seem to be able to survive very well on normal skin but they are readily isolated from the human alimentary tract and vagina [1,2].

Of importance to clinical candidiasis, eucaryotic *Candida* spp. are resistant to antibacterial antibiotics [1,2] and they are able to increase in numbers in the alimentary tract and vagina during therapy with broad-spectrum antibacterial antibiotics [1,2,15,20-22]. Increased populations of *Candida* spp. in the alimentary and vaginal tracts that occur during antibacterial antibiotic therapy increase the incidence of mucosal [2,6,12] and systemic candidiasis [1-12]. Not only is *Candida albicans* resistant to antibacterial antibiotics, but many *Candida* spp. (e.g., *Candida glabrata)* are resistant to antimycotic drugs [23]. Some strains of *C. albicans* that resist azole antimycotic drugs are emerging in the populations of immunodeficient patients [24].

C. albicans comes close to being an almost perfect human parasite because of its unique capacities to colonize, infect, immunize (i.e., stimulate both antibody- and cell-mediated immune responses in humans), and persist on mucosal surfaces of most humans world-wide [1,2,6,20,21]. The wide-spread presence of serum and secretory antibodies to *C. albicans* [25] and sensitized T-lymphocytes, that respond to *C. albicans* antigens [26] attests to the highly infectious nature of this microorganism for most humans and provides further proof that most humans have been naturally immunized by prior contacts with *Candida* spp.

Chronic colonization, subclinical infection, and natural immunization of humans with *Candida* spp. raise important questions about human resistance to candidiasis. Is natural immunization with *Candida* spp. responsible for the fact that the vast majority of adult humans do not have serious clinical problems with candidiasis? Or, do other innate and acquired immune and non-immune factors play a role in the recognized resistance of most adults to candidiasis?

The persistence of *Candida* spp., especially *C. albicans*, on mucosal surfaces of the alimentary tract and vagina [1,2] and on hands of medical personnel [5,10,12] accounts for its ease of transmission among infants and adults. Most infants come in contact with, and are colonized by, *C. albicans* during vaginal birth [1,2,27]. Candidiasis, in most healthy neonates, is subclinical or mild (diarrhea, diaper rash, etc.) [27]. Such benign encounters with *Candida* are thought to play a key role in stimulating the antibody- and cell-mediated immune responses to *Candida* spp. that are evident in most humans [1,2,25,26]. In fact, the interaction of *C. albicans* with the human immune system is so common that skin tests with *Candida* antigens are frequently used to assess the status of their cell-mediated immune responses [26] and antibodies to *C. albicans* antigens are so common in human sera that the reliability of using antibodies to *C. albicans* in serology tests for the diagnosis of systemic candidiasis is diminished [25]. The antibody- and cell-mediated immune responses evoked by *C. albicans* in humans are apparently not sufficient to completely expel *C. albicans* from the mucosal (alimentary tract and vagina) surfaces of a large percentage of humans since most remain chronically-colonized with *C. albicans* (albeit in low numbers relative to number of bacteria present in the normal flora of humans) throughout their life [1,2].

It is often hypothesized that antibody- and cell-mediated immune responses, and the acquired immunity most humans develop after natural immunization with *Candida* spp. are responsible for protecting the vast majority of *Candida*-colonized adults from candidiasis. Although innate and acquired immune responses (intact epithelial cells, phagocytic cells, acquired antibody- and cell-mediated immunity) do play an important role in resistance to mucosal and systemic candidiasis it is also well known that humans who manifest both antibody and cell-mediated immunity to *C. albicans* antigens can be infected with *C. albicans* when the number of *C. albicans* in their alimentary tract is increased [1,2] or when indwelling catheters become contaminated with *Candida* spp. [1,2]. For example, an immunocompetent person who drank a culture of *C. albicans* (10^{12} *C. albicans*/ml) deve-

loped life-threatening systemic candidiasis (candidemia and uremia) and had to be treated with antifungal antibiotics [28]. Conversely, many immunodeficient patients do not have problems with candidiasis until their bacterial flora is disrupted by oral antibiotic therapy and *Candida* overgrowth on mucosal surfaces occurs [1-12]. Humans or research animals who are treated with broad spectrum antibiotics which upsets the diversity of the normal enteric flora and allows *C. albicans* to increase in number in the alimentary tract often develop candidiasis (oral, esophageal, and/or vaginal) [1,2,10,12,15,21]. A good deal of data provides strong support for the fact that the anti-*Candida* activities of the microbial flora play a key role in protecting either *Candida*-naive, immunocompetent or immunodeficient hosts from candidiasis. Thus, a large number of immunocompetent and immunodeficient hosts remain free of clinical candidiasis as long as their *Candida*-inhibitory bacterial flora remains intact and their innate and acquired immune systems are not substantially diminished through disease or therapy.

The anti-*Candida* activity of the enteric bacterial flora plays a very important role in resistance to candidiasis because it is a "first-line" defense mechanism that can suppress the growth of commensal *Candida* spp., and thereby interfere with the adherence and growth of *Candida* spp. on epithelial surfaces.

In addition to prophylaxis, the intestinal flora can also exert biotherapeutic effects against candidiasis. Simply stopping oral antibacterial antibiotic therapy, which allows the restoration of a complex bacterial flora often results in dramatic improvements in antibiotic-induced candidiasis [1,2,21]. Unfortunately, we still know very little about the intestinal microbes that are responsible for these probiotic and biotherapeutic effects against candidiasis. More importantly, the mechanisms that intestinal and vaginal probiotic microbes use to bring about the suppression of the opportunistic yeasts are still far from clear.

A number of bacterial species have the capacity to inhibit *C. albicans in vitro* [29-31]. Antibacterial antibiotics, which selectively inhibit anaerobic intestinal bacteria are frequently associated with *C. albicans* overgrowth [15]. *Saccharomyces boulardii* has been shown to inhibit disseminated candidiasis (translocation of *C. albicans*) in mice [32] and *Lactobacillus acidophilus* has shown efficacy in the biotherapy of *Candida* vaginitis [33]. *In vivo* studies to assess the impact of specific microbes on candidiasis have been few in number and most have been carried out in gnotobiotic rodents; however, *Escherichia coli*, lactobacilli, oral streptococci and bifidobacteria have all been shown to be capable of suppressing the growth of *C. albicans* in the alimentary tract of gnotobiotic rodents [8,34-41]. It was recently shown that probiotic lactic acid bacteria could protect adult, neonatal, and adult gnotobiotic, immunodeficient mice from candidiasis [40]. Thus, it is evident that a variety of intestinal microbes, in pure culture, can not only inhibit the growth of *Candida* spp. *in vivo* and *in vitro*, but they can also protect immunocompetent and immunodeficient hosts from candidiasis. The latter data contrasts with evidence that a diversity of bacterial species are needed for efficacy of probiotics [12].

A variety of mechanisms have been evoked to explain the anti-*Candida* activity of the probiotic, anti-*Candida* microbes. Nutritional competition, blocking receptors for *Candida* spp. adhesins on epithelial cells, production of anti-*Candida* compounds, increasing intestinal peristalsis, increasing intestinal epithelial cell renewal rates, alteration of pH and the production of an anaerobic oxidation-reduction potential (*C. albicans* is an aerobic microbe) have all been proposed as mechanisms that probiotic bacteria use to inhibit pathogens on mucosal surfaces. The capacity of the bacterial flora to stimulate innate and acquired immune systems in the host and activate phagocytic cells is also thought to play a role in the inhibition of *Candida* spp. by probiotic bacteria [40]. Very likely, because of the recognized complexity of the aerobic and anaerobic normal bacterial flora, all of the above factors are involved in the suppression of *Candida* spp. on mucosal surfaces.

The inhibition of *Candida* spp. by probiotic bacteria in the alimentary and vaginal tracts represents a key, first-line defense against mucosal and systemic candidiasis. At times the anti-*Candida* activity of the microbial flora, which prevents overgrowth of *C. albicans* on mucosal surfaces, may be more important in preventing candidiasis than innate and acquired immune mechanisms [2,10,12]. The role of a probiotic is to supplement this first-line of defense. As long as the bacterial flora can prevent or inhibit the growth of *C. albicans* on mucosal surfaces, most adult men and women will not suffer from clinical candidiasis; however, whenever alterations occur in the bacterial flora, either through therapy with antibiotics or disease, *Candida* spp. can increase in number which enhances the possibility that they can cause a variety of clinical diseases (diarrhea, cutaneous, mucocutaneous, oroesophageal, and even systemic candidiasis and lethality); the form and severity of the candidiasis that develops appears to depend upon the immunocompetence of the host [1,2,10,12].

Further research is needed to identify the probiotic, biotherapeutic, anti-*Candida*, microbes and the mechanisms they utilize to control the growth of *C. albicans* on mucosal and cutaneous surfaces. The fact that inhibition of intestinal bacteria with antibacterial antibiotics and *Candida* overgrowth are so often the prime predisposing factors in candidiasis [1,2,10,12] should be sufficient impetus to learn more about how to retain or augment the anti-*Candida* activities of the intestinal bacteria with probiotics. Such procedures could suppress, or eliminate *Candida* spp. from the alimentary and vaginal tracts and thereby prevent clinical candidiasis.

CONCLUSIONS

Most adult humans have continuous contact with *Candida* spp. throughout their lifetime. The fact that antibody- and cell-mediated immune responses to *Candida* antigens are so common in humans suggests that most adults have been naturally immunized by subclinical encounters with *Candida* spp. It is also well recognized that antibacterial antibiotics can enhance the susceptibility of the host (even naturally immunized and immunocompetent hosts) to candidiasis. The anti-*Candida* activity of the bacterial flora is an "acquired" host defense mechanism that plays a very important "first-line of defense" role in protecting humans against candidiasis since the bacteria in the intestinal flora not only can inhibit the growth of *Candida* spp., but they can also interfere with the capacity of the pathogenic yeasts to adhere to epithelial tissues.

Our lack of knowledge about the probiotic intestinal and vaginal microbes that inhibit *Candida* spp. and about the mechanisms they use to suppress the opportunistic yeasts in the alimentary tract and vagina has hindered our capacity to maximize the use of probiotic bacteria

for the prophylaxis and therapy of candidiasis. The fact that *Candida* spp. have shown increased resistance to antifungal agents and that there is still a reluctance to use antifungal agents for prophylaxis in at-risk patients, provides new impetus and relevance for studies on *Candida*-inhibiting probiotic bacteria. Probiotic bacteria could be a very practical and innocuous way to reduce the expanding numbers of candidiasis cases, and the increasing resistance of *Candida* spp. to antifungal agents. The powerful anti-*Candida* effects of probiotic bacteria should not be taken lightly or ignored any longer.

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