Effects of Probiotics against Candida Infections and Their Potential Use in Vulvovaginal Candidosis

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Abstract

Candida species are frequently found in the human microflora of the oral, intestinal and vaginal mucosa, where they usually live as commensals and interact with microbial communities and immune cells for the development and homeostasis of the immune response. However, many Candida spp. also act as opportunistic pathogens and are capable of causing a wide range of fungal diseases, among which vulvovaginal candidosis is very frequent. In recent years, probiotics have been investigated in several pathologic conditions as an approach to modulate the microbiota, and in this regard, they have also been proposed as an adjuvant therapy in prevention and treatment of vulvovaginal candidosis. In this review we focus on mechanisms of Candida tissue invasion and of host defense and discuss the role of probiotics in prevention and management of vulvovaginal candidosis. Anti-Candida potential of Lactobacillus acidophilus W22 and Lactococcus lactis W19, which are contained in a recently introduced multi-strain symbiotic, is discussed.

Keywords: Vulvovaginal Candidosis; Probiotics; Multistrain; Lactobacillus acidophilus; Lactococcus lactis

1. Introduction

Humans live in association with immense populations of bacteria, viruses and fungi. (Hoffmann et al. 2013) Among these, Candida species are frequently found in the human microflora, as they are capable of colonizing the oral, intestinal and vaginal mucosa, as well as the skin of healthy individuals (Sparber and Leibundgut-landmann 2015).

In these compartments, they usually live as commensals and interact with microbial communities and immune cells for the development and homeostasis of the immune response. However, many Candida spp. also act as opportunistic pathogens and are capable of causing a wide range of fungal diseases. (Sparber and Leibundgut-landmann 2015) Superficial (or mucosal) Candida infections are very frequent indeed, and include oral candidosis and vulvovaginal candidosis (VVC). While in the

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immunocompromised host, fungi – including *Candida* spp. – can overcome the epithelial barrier and enter the bloodstream, causing invasive fungal infections, which are associated with significant morbidity and mortality in these patients (Soysal 2015).

Probiotics are live micro-organisms which produce a health benefit when administered in certain amounts to the host (Sanders et al. 2013), while prebiotics are dietary non-digestible ingredients that selectively stimulate the growth and activity of one or a limited number of bacterial species of the intestinal flora. The combination of probiotics and prebiotics forms a symbiotic, which is useful to increase the survival of the probiotic organisms because it immediately makes its substrate available for fermentation (Rossi, Rossi, and Fassio 2015; Manzotti, Heffler, and Fassio 2014a; Manzotti, Heffler, and Fassio 2014b).

In recent years, probiotics have been investigated in several pathologic conditions as an approach to modulate the gastrointestinal microbiota (O’Mahony et al. 2005; Rousseaux et al. 2007; Bertelsen et al. 2013; Wald and Rakel; Mitsuyama and Sata 2008; Seksik et al. 2008; Guandalini, Cernat, and Moscoso 2014). They showed promising results in a multitude of gastroenteric and extra-intestinal pathologic conditions (Ciorba 2012; Eigenmann 2013; Vandenplas, Huys, and Daube 2014; Sanders et al. 2013).

In this regard, probiotics have also been proposed as an adjuvant therapy in prevention and treatment of vulvovaginal candidosis VVC.

2. Vulvovaginal Candidosis

It has been established that *Candida* species colonize the estrogenized vagina in about 20% of women, and that this ratio increases up to 30% in late pregnancy and in immunosuppressed patients.

*Candida albicans* infection of the estrogenised vagina and vestibulum is defined as vulvovaginal candidosis (VVC), which can also extend to the outer sides of the labia minora, the labia majora, the intercrural region and the perineal region (Mendling 2015).

From epidemiological studies, we know that about 3 out of 4 women are expected to have at least one episode of VVC during their reproductive age, while half of them will have two or more episodes (Dovnik et al. 2015).

In the majority of cases, VVC presents with burning pain and pruritus of the vulva, dysuria or even dyspareunia in more severe cases. (Anderson, Klink, and Cohrssen 2004) Sporadic episodes of mild *C. albicans* infection are defines as uncomplicated VVC, while complicated VVC is characterized by The course of VVC is usually uncomplicated, but complicated VVC (in case of severe infection, infection by other species than *C. albicans*, during pregnancy, associated with other conditions such as immunodeficiencies or diabetes. recurrent VVC (RVVC) must be taken into account. Recurrent VVC (RVVC) is also a form of complicated infection and is defined as four or more episodes of VVC per year; *C. glabrata* and other *non-albicans* forms are responsible for 10 to 20% of these cases (Dovnik et al. 2015)
Clinical signs of VVC comprehend local inflammation (edema and erythema of the vulva and the vagina) accompanied by vaginal discharge that may be minimal, watery or cheese-like (Dovnik et al. 2015).

A number of different host factors, such as local defense mechanisms, genetic polymorphisms, immunological status, serum glucose levels, use of antibiotics, psychosocial stress and estrogen levels can influence the risk of Candida colonization in the female urogenital tract. Although the pathogenesis of VVC remains controversial, it seems that Candida overgrowth is facilitated when the balance between the microorganisms existing in the vaginal microbiota is disrupted (Falagas 2006).

2.1 Recurrent vulvovaginal candidosis

In the case of RVVC, the clinical condition can be even more challenging, as the symptoms are recurring (four or more episodes per year) and the infection could be increasingly refractory to medical treatments (Sobel et al. 2004). Long-term maintenance therapy with fluconazole may be of help, as it could lengthen the asymptomatic period between recurrences, but this is frequently unsatisfactory anyway (De Bernardis et al. 2015).

Among the proposed causes of antimicrobial failure in RVVC, inability to restore bacterial community homeostasis must be considered.

2.2 Treatment regimens

For treating uncomplicated VVC a short-term local therapy with azoles (no single drug seems to be significantly superior to the others) or a single-dose oral treatment with fluconazole (150 mg) is usually recommendend: these regimens can achieve resolution of VVC in about 90% of cases (Dovnik et al. 2015).

On the other hand, complicated VVC requires prolonged treatments, such as 3 doses fluconazole (72 hours apart one from another) or a one-week course of local azoles (Dovnik et al. 2015). Chances of success decrease in treating infections supported by Candida non-albicans species.

3. Epithelial Immunity and Mechanisms of Infection in VVC

Low burdens of C. alibicans, most of which in yeast form, are not pathogenic to the host and can be tolerated. In this context, C. alibicans can trigger some pattern recognition receptors (PRRs) which seem to differ from classic ones (e.g. Toll-like receptors, C-type lectin receptors) and are still to be elucidated. Their activation leads to mechanisms of epithelial damage protection and disease prevention (Naglik, Richardson, and Moyes 2014).

Conversely, increased burdens of C. alibicans in form of hyphae, lead to activation of innate immunity, which in turn leads to secretion of cytokines and chemokines and to recruitment of adaptive immunity effector cells. Among these, a prominent role is attributed to Th1 and Th17 lymphocyte subpopulations (De Bernardis et al. 2015; Sparber and Leibundgut-landmann 2015) (Fig. 1).
3.1 Mechanism of Candida invasion of vaginal epithelial cells

Among Candida spp., C. albicans can be found in about 50% of individuals in the general population and is the principal responsible for fungal mucosal infections. In healthy individuals, epithelial cells constitute the first line of defense against fungal invasion.

C. albicans ability to switch among two morphological forms – yeast and hyphal – is crucial in order to gain pathogenicity and virulence. Several proteins unique to the hyphal form, such as hyphal wall protein-1 (Hwp1p) and agglutinin-like sequence 3 (Als3p), have been recognized able to promote adherence to epithelia and tissue invasion. Moreover, in the hyphal form, other virulence attributes such as biofilm formation and hydrolytic enzyme production, are increased. On the other hand, loss of these characteristics has been associated with non-invasive and avirulent behavior.

Two distinct, yet complementary mechanisms involved in host cell invasion by C. albicans have been identified so far: these are fungal-induced endocytosis and active penetration. While endocytosis seems to play a major role in early stages of invasion (first 4 hours), active penetration is the predominant route of epithelial invasion. In both cases, hyphal form seems to be necessary in order to penetrate tissues.

4. The Role of Probiotics in Treatment and Prevention of VVC

The vaginal microbiome plays an important role in health and disease, including VVC. In both conditions, here Lactobacillus is the dominant genus.
Probiotics and prebiotics, which are capable of influencing the composition of the microbiome, could help to prevent candidosis thanks to their multiple mechanisms of action.

Probiotics are indeed capable of a variety of effects, including regulation of intestinal microbial homeostasis, vital competition with other pathogens trying colonize and infect the mucosa, immunomodulation of local and systemic responses, maintenance and improvement of the epithelial barrier function (table 1), (Boirivant and Strober 2007) all of which have a role in preventing Candida excessive proliferation and/or infection of host tissues.

Table 1 Immunological and non-immunological effects of probiotics (modified from (Allergy Therapeutics Italia 2015)).

<table>
<thead>
<tr>
<th>Immunological effects</th>
<th>Non-immunological effects</th>
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<tr>
<td>Macrophages activation</td>
<td>Vital competition against pathogens</td>
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<tr>
<td>Antigen presenting cells stimulation</td>
<td>Bacteriocines production</td>
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<tr>
<td>Increase of IgA secretion by B lymphocytes</td>
<td>Elimination of superoxide radicals</td>
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<tr>
<td>Modulation of immune response and food</td>
<td>Strengthening of epithelial barrier</td>
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<tr>
<td>tolerance induction</td>
<td></td>
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<tr>
<td>Modification of cytokine secretion pattern</td>
<td>Pathogen-derived toxin inactivation</td>
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<tr>
<td>(variable depending on probiotic strain)</td>
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In these days, probiotics are commonly used in a variety of clinical conditions, even without medical advice or prescription; it is no surprise, therefore, that many women who suffer from VVC and/or RVVC already use them.

In a survey, 73% of 1117 women in the age range 18–70 years self-reporting having had symptoms suggestive of VVC, declared that they used Lactobacillus probiotics for prevention and treatment of post-antibiotic vulvovaginitis in 43% and 40% of cases, respectively (Pirotta, Gunn, and Chondros 2003).

Several studies, both in vitro and in vivo, evaluating the effectiveness of probiotics against Candida infections have been carried out.

4.1 In vitro studies

Probiotics have been showed to protect from Candida infection, but the underlying mechanisms are still not clearly understood. The adhesion of vaginal Lactobacilli to epithelial tissue, thus constituting a sort of barrier, represents an important step against undesirable microbial colonization (Parolin et al. 2015).

A study by Osset et al. on vaginal exudates collected from 115 women showed that some H2O2-producing Lactobacillus strains (8 of 15) were capable of inhibiting adhesion of C. albicans to vaginal epithelial cells; some of them were also capable of inhibiting Candida growth in liquid assays. (Osset et al. 2001)

In a different study, Strus et al. demonstrated that inhibition of Candida growth was not solely related to H2O2-production, as extended activity, demonstrable after 24 hours, was shown by the
non-H2O2 producing *L. plantarum* (Strus et al. 2005) In the same study, the Authors suggest that mixtures of different *Lactobacillus* strains cooperate synergistically using many different metabolites.

Boris *et al.* observed that the adherence of *C. albicans* to the vaginal epithelial cells was greatly decreased in presence of *L. acidophilus*, compared to the rate of adherence observed when only *Candida* was present (Boris et al. 1998).

*L. plantarum* 319, *L. rhamnosus* IMC 501 and *L. paracasei* IMC 502 were shown to inhibit *Candida* adherence to HeLa cells, in a strain-dependent manner (Coman et al. 2015) It is of interest that in this study, each probiotic could inhibit the adherence of Candida non-albicans strains too, suggesting that this effect could be carried out by barrier and interference mechanisms (Coman et al. 2015).

Other specific *Lactobacilli*-produced factors seem to play a role against *Candida* infections: a biosurfactant produced by *L. acidophilus* RC-14 (‘surlactin’) has been showed able to decrease the adherence of *Candida* cells on silicone rubber by 50%, while ‘pentocin TV35b’, a bacteriocin-like peptide isolated from *L. pentosus*, inhibited the growth of *C. albicans* (Velraeds et al. 1998; Okkers et al. 1999).

Recently, a metabolomics analysis of *Lactobacillus* strains isolated from vaginal swabs of healthy premenopausal women was conducted. It showed that different strains were capable of exerting *in vitro* activity against *Candida* spp. by exclusion, competition and displacement mechanisms, and that the most active strains in reducing pathogen adhesion were capable of all three of them. (Parolin et al. 2015) The spectrum of activity against different *Candida* species varied among the probiotic strains; in particular, among those considered in this study, *L. crispatus* BC1 and *L. vaginalis* BC15 exhibited the best anti-*Candida* profile, being effective also against *C. tropicalis* and *C. glabrata* (Parolin et al. 2015).

### 4.2 In vivo studies

Studies evaluating the effectiveness of probiotics in prevention and/or treatment of VVC have shown conflicting results. This is not surprising as a number of methodological lacks are known to affect the majority of clinical studies involving probiotics, such as small participants number, no placebo control groups, use of different probiotic strains, different administration scheme, variable duration and dosage (Dovnik et al. 2015).

A review by Falagas *et al.* published in 2006 concluded that the results of some clinical trials supported the effectiveness of *Lactobacilli*, especially *L. acidophilus*, *L. rhamnosus* GR-1 and *L. fermentum* RC-14, administered either orally or intravaginally in preventing the colonization and infection of the vagina by *C. albicans*, while the results of a small number of other clinical trials did not corroborate these findings (Falagas 2006) The Authors of this review concluded that empirical use of probiotics may be considered in women with frequent recurrence of VVC, especially in those cases in which antifungal agents are contraindicated (Falagas 2006).

A randomized placebo-controlled trial evaluating the effectiveness of probiotics in restitution of normal vaginal microflora after VVC demonstrated increased probability of normal vaginal
microflora in the treatment group (daily capsules containing *L. reuteri* RC-14 and *L. rhamnosus* GR-1 for six weeks) compared to the placebo group (Vujic et al. 2013).

Two different studies evaluated the addition of probiotics to itraconazole therapy in patients with VVC: a significant increase of culture-free patients was observed if the probiotics treatment was carried on for 4 weeks, (Martinez et al. 2009) while no effects were noticed if it lasted six days (Witt et al. 2009).

*In vitro* and *in vivo* studies about anti-*Candida* activity of other non-*Lactobacillus* strains are still lacking.

Even in absence of a strong evidence for probiotics use in VVC, a recent guideline considers this approach encouraging and deserving further investigations (Mendling 2015).

In this regard, probiotics could be considered as an adjuvant treatment (together with the antimicrobials) in VVC, while clinical trials directly comparing efficacy of standard antimicrobial therapies versus probiotics are lacking.

Interestingly, probiotics could result even more beneficial in those cases of complicated VVC supported by *Candida* species which are not susceptible to antimicrobial therapies (most of which are represented by *C. non-albicans*). As demonstrated by some *in vitro* studies, probiotics in some cases are capable of inhibiting *Candida* by barrier and interference mechanisms that are not influenced by *C. strain* or classic drug-resistance capabilities (Coman et al. 2015; Parolin et al. 2015).

5. *Identification of Probiotic Strains with In Vitro Anti-Candida Activity*

Several different probiotic strains have been tested *in vitro* for *Candida* inhibition potential. Among these, *L. acidophilus* W22 and *Lactococcus lactis* W17 have been selected for their capabilities (Allergy Therapeutics Italia 2015).

Single strains were added to a tube containing 10 ml of liquid bacterial growth medium MRS. In the same tube the *Candida* strains were added. After incubation, the growth of both probiotic strains and the *Candida* were determined in each tube. The anti-*Candida* activity of the probiotic bacteria was measured as a decreased growth rate of *Candida albicans* compared to the control (= 0% inhibition) (Allergy Therapeutics Italia 2015).

*L. acidophilus* W22 showed an inhibition of growth of 94.9% for *C. albicans* 14053 and even higher results for other *Candida* strains, while *Lactococcus lactis* W19 showed an inhibition of *C. albicans* growth of 85.5% (Fig. 2).
Fig. 2. Inhibition of Candida strains by L. acidophilus W22 and Lc. lactis W17 (modified from (Allergy Therapeutics Italia 2015)).

From these results, it seems that L. acidophilus W22 and Lactococcus lactis W17 have a significant capability of inhibiting C. albicans in vitro and this could lead the way to their employment as therapeutic adjuvant in VVC.

In a recent study, the effect of several probiotic strains on variation of trans-epithelial electrical resistance (TEER) was evaluated. (Besseling-van der Vaart et al. 2015) TEER is an electrophysiological in vitro measurement of the movement of ions across an epithelium barrier by means of paracellular transport, and can be used as an index of strength for the epithelial barrier. It was shown that Lc. lactis W17, and to a lesser extent L. acidophilus W22 were able to significantly improve TEER. This effect can be attributed to the strengthening of intercellular tight junctions and could be important to limit epithelial tissues invasion by pathogens, including Candida spp.

6. Selection of Strains for a Symbiotic Formulation with Anti-Candida Activity

It is known that a single probiotic strain has the capacity to accomplish just a few of the functions attributed to probiotics, while a broad reading of the literature reveals that different probiotics have been associated with different effects, mostly related to their unique capacities to express specific surface molecules (Marco, Pavan, and Kleerebezem 2006; Boirivant and Strober 2007).

For this reason, a well-balanced multi-strain probiotic formulation could be superior to a single-strain probiotic in achieving the desired result (Besseling-van der Vaart et al. 2015).
A recently introduced symbiotic formulation of four strains (*Lactococcus lactis* W19, *Lactobacillus acidophilus* W22, *Bifidobacterium lactis* W51, *Lactobacillus plantarum* W21) together with the prebiotic inulin has been recently marketed for the restoration of gut microflora. (Besseling-van der Vaart et al. 2015) Given the anti-*Candida* properties of some of the probiotic strains therein contained, this formulation as an interesting potential adjuvant therapy in prevention and/or treatment of VVC and RVVC.

### 7. Conclusion

VVC is a very common and bothersome clinical condition. Given the broad range of effects that probiotics are capable of, in order to restore the vaginal microbiome, they have been increasingly studied – both *in vitro* and *in vivo* – in the context of VVC. Even if a strong evidence cannot be determined from studies published so far, results are encouraging. The use of multistrain probiotic preparations, and possibly the addition of prebiotics in order to stimulate specific strains growth, could represent a step forward in this direction. A recently introduced symbiotic formulation of *Lactococcus lactis* W19, *Lactobacillus acidophilus* W22, *Bifidobacterium lactis* W51, *Lactobacillus plantarum* W21 together with inulin has shown interesting capabilities of strengthening the epithelial barrier and inhibiting *Candida* growth. Its potential as therapeutic adjuvant in the prevention and treatment of VVC and RVVC is intriguing and should be assessed in further studies.

### Conflict of Interest

Filippo Fassio received medical consultancy fees from Allergy Therapeutics Italia.

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