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Review

Use of Probiotics for Oral Candidiasis: State of the Art and Perspective. A Further Step Toward Personalized Medicine?

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Abstract

Oral candidiasis is an opportunistic infection conventionally treated with antifungal drugs. However, the increasing number of fungal infections, parallel to the rising conditions sustained by non-albicans species, pose critical issues related to escalating drug resistances differently acquired by different species. Meanwhile, the knowledge of the interplay between oral microbiota and its host suggests alternative antifungal therapies based on the administration of probiotics. Probiotics are live microorganisms beneficial to the host, and literature reports consistent evidence for their use to treat gut diseases. The present work aimed to overview the primary mechanisms through which probiotics act against Candida species and the current status of knowledge on their use in clinical practice, particularly concerning oral candidiasis.

Keywords: candida species; oral candidiasis; dentistry; dysbiosis; functional food; infectious diseases; microbiota; probiotics; therapy

1. Oral Candidiasis

Oral candidiasis is an opportunistic infection of the oral mucosa, sustained by various yeasts of the *Candida* genus overgrowing and becoming virulent under peculiar conditions of oral dysbiosis, secondary to changes in the oral microbiome diversity and composition driven by predisposing systemic and local factors [1].

Over 150 Candida species have been recognized to date. *C. albicans* is the most frequently associated with human infections. It is a dimorphic Gram-positive yeast, not acid-resistant, saprophyte in 40–75% of healthy humans [2] and responsible for over 80% of human candidiasis [3]. Furthermore, the number of infections sustained by non-albicans species (NACs) is increasing globally, presumably due to their different susceptibility and responses to drugs compared to *C. albicans*, thus leading to the selection of candidiasis supported by drug-resistant NAC [4]. The NAC species most frequently involved in human candidiasis are *C. parapsilosis, C. glabrata, C. tropicalis, C. krusei, C.dubliniensis, C. lusitaniae, C. guilliermondii, C. rugosa, C. famata,* and *C. kefyr* [5].

Each species is distinguished and identified from the others by molecular techniques—which recognize their specific genome- or cultural methods based on the morphology of the colonies, the type of sugar fermentation, and the serological reactions [1,5]. Apart from the conventional microbiological cultures, which require up to two days for fungal detection, thus being time-consuming, the molecular-based methods identify each strain by their specific 16s rDNA, 19s rDNA, or internal transcribed spacer (ITS) regions, thus allowing to distinguish portions of the Candida genome and identify each strain according to its species-specific DNA sequences. Although ultrasensitive, specific, rapid, and accurate, these methods are costly and require trained laboratory personnel to perform the test correctly [6]. Hence, a different method advantageously used to rapidly detect and accurately identify fungal pathogens in the clinical setting is the "matrix-assisted laser desorption/ionization time-of-flight mass spectrometry" (MALDI-TOF MS). Through the MALDI-TOF MS process, a wide variety of microorganisms directly from the selective medium used to isolate them are rapidly and precisely identified based on their specific peptide mass spectra, matched with the spectra stored in an integrated database, which automatically identify and report the specific strains [7,8]. Apart from the high initial cost of the MALDI-TOF equipment, the method is rapid, sensitive, and economical in terms of the labor and costs involved. The main limitation is that identification is possible only if the spectral database contains peptide mass barcodes of the specific genera/species/subspecies/strains [8].

C. albicans dimorphism is the reversible phenotypic switch from blastospores -peculiar in the absence of active infections- to hyphae or pseudohyphae, occurring under predisposing local and metabolic factors in case of active infections [9]. These events contribute to extreme antigenic polymorphisms, which allow Candida to elude the host immune responses [10,11]. Furthermore, these yeasts can also adhere to the oral mucosa, dental surfaces, and abiotic surfaces, as dentures and orthodontic appliances, through Candida adhesins [12–14], and can invade the epithelia thanks to cytolytic enzymes [13]. At the same time, in specific con-

ditions, some strains can also produce carcinogenic metabolites [10]. Last, while the planktonic find is usually in asymptomatic/carrier status, the biofilm organization of aggregates or co-aggregates Candida species is responsible for the increase in virulence and drug-resistances [15].

The variety of clinical forms and severity is strictly related to the host factors and the virulence of the strains [16]. The clinical forms can be mainly classified into acute oral infections, such as pseudomembranous, erythematous, and angular cheilitis, and chronic candidiasis, such as atrophic, hypertrophic, denture stomatitis, and median rhomboid glossitis [1,17].

The infection is conventionally eradicated with antifungal drugs, mainly azoles, polyenes, and synthetic drugs derived from echinocandins. While these latter have direct effects on the fungal wall, polyenes and azoles, in addition to altering the fungal plasma membrane by interfering with the synthesis and positioning of ergosterol, can also interfere with the cholesterol synthesis of the eukaryotic cell wall, thus presenting hepatotoxic and nephrotoxic effects on the host. These side effects and drug resistance phenomena must not be underestimated in prolonged or recurrent use of these drugs [18–20].

All these events pose the research towards the experimentation of therapies alternative to conventional drugs, with lesser side effects and harmful repercussions on the general and local health of the patients [1].

Moreover, the success of any antifungal therapy first requires the adjustment of local and systemic conditions predisposing the fungal infections, mainly unsanitized or not-fitting dentures and lack of oral hygiene [21], as the principal local factors, and vitamin and iron deficiencies, immunological disorders, uncontrolled diabetes, and steroidal or immunosuppressive systemic therapies, as systemic factors [22–24]. Otherwise, when oral candidiasis recurs, each consecutive episode may predispose to higher risks of aggressiveness and dissemination, related to fatal events in half of the cases, secondary to candidemia and systemic dissemination of the infections from the mouth to the bloodstream [25].

A novel approach to eradicating fungal and bacterial infections involves using probiotics, based on the capability to re-balance a healthy microbiota. This overview aims to report the primary mechanisms through which probiotics act against Candida species and the current status of knowledge on their use in clinical practice.

2. Probiotics

Probiotics are "live microorganisms which, when consumed in adequate amounts, confer health benefits on the host" [26–28].

The study of the interrelationships between beneficial and harmful bacteria and the host has been progressively recognized and culminated with the research on the human microbiota, as the whole microbial community coliving with multicellular eukaryotic organisms, as humans and other living beings [29]. Then, the knowledge of the essential sharing of vital functions by humans and microbes, which co-exist and co-evolve together during each period of life, from birth to death, and during the succession of generations, led to coining the term "holobiont", as a superorganism made by complex eukaryote organisms and all the microorganisms -not only bacteria but also fungi, viruses, and protozoan- and their genetic material (both eukaryote genome and microbiome), symbiotically living and existing one thanks to the other [30,31]. Therefore, while each host and its typical microbiota live in balance and benefit, each disruption, or dysbiosis, may cause consequences on their wellbeing, expressed by the onset of diseases of various origins and severity.

The administration of beneficial bacteria during diseases associated with or sustained by dysbiosis to replace the prevaricating harmful microbes was first suggested by the Nobel Prize Winner Eli Metchnikoff and the pediatrician Henry Tissier a century ago. They discovered the beneficial role of the administration of bifid bacteria in restoring healthy gut flora in children suffering from diarrhea [32,33].

Since then, and still today, probiotics have been commonly used as dietary interventions to rebalance the gut microbiome to manage a series of gastrointestinal disorders. Among the indication, gut dysbiosis secondary to prolonged broad-spectrum antibiotic therapies, which also deplete saprophytic bacteria, thus favoring the proliferation of pathogenic species, mainly responsible for diarrhea and, consequently, loss of electrolytes and lack of nutrients usually absorbed by a healthy gut mucosa with the appropriate microbiota. Furthermore, probiotics also contrast *Clostridioides difficile* infections and are used to manage lactose intolerance and prevent necrotizing enterocolitis in preterm infants [34–37].

However, neither in Europe nor the United States, probiotics are considered drugs (but "food supplements"). Hence their regulatory status and marketing are less controlled than pharmaceutical products, and the experimental demonstration of the effectiveness of each formulation, significantly differing from each other for quality and variety of live microorganisms included, do not always have the same efficacy in the clinical practice, as well their viability at the body site of action. Recently the American Gastroenterological Association (AGA) released clinical practice guidelines for the appropriate use of specific probiotics in the context of specific gastrointestinal diseases in specific subjects (neonates, children, and adults), thus proposing strong or conditional recommendations for each peculiar clinical and patient's conditions [35].

Other than the applications in intestinal diseases to restore the intestinal flora, probiotics are also currently used in other extra-intestinal diseases and for the health of other organs and systems, such as example, the respiratory tract [38], the skin [39], the urogenital tract [40], and the oral cavity [41–43].

3. Probiotics in Oral Candidiasis: Mechanisms of Action

Since infections sustained by Candida species take advantage of local dysbiosis, both transient, as after prolonged therapy with broad-spectrum antibiotics, and chronic, as in immune deficiencies, it is reasonable to approach them by administering beneficial bacteria to reestablish the local flora, which can compete against Candida colonization and infection.

The most used probiotics belong to Lactobacillus spp., Bacillus spp., Bifidobacterium spp., and Saccharomyces spp. [17]. Moreover, the antifungal mechanisms of action, varying according to the species and strains used with different encouraging effects, have been reported below.

3.1 Lactobacilli

Lactobacilli are among the most studied bacteria and are used as probiotics to rebalance the microbiota of the orodigestive and genitourinary tracts or to prevent their dysbiosis.

Lactobacillus spp. are facultative-anaerobic rod-shaped Gram-positive bacteria producing as the final metabolites a series of organic acids such as lactic acid (the main product of homofermentative lactobacilli) and acetic and phenyl lactic acids (as those produced by the heterofermentative lactobacilli) [44,45]. Over 200 species of the *Lactobacillus* genus have been recognized to date, highly varying in phenotype, ecology, and genotypic level; their complex taxonomy has been variously rearranged until the last one by Zheng *et al.* [46] who have reorganized 26 phylogenetic groups.

The lactobacilli most used as probiotics are various strains of *L.reuteri*, typically intestinal, and *L.casei* and *L.acidophilus*, also present in the cervical microbiota. These species have also been found in saliva in subjects with caries and without [47]. However, the literature about oral microbiota seems to favor the hypothesis of the protective role of lactobacilli against fungal infections [48] and attributes greater cariogenic properties to Candida than to lactobacilli [49].

Different lactobacillus species exert different anticandidal activities, favored by various mechanisms and metabolites, summarized as follows.

3.1.1 Creation of an Environment Hostile to the Growth of Candida

Lactobacilli are aciduric facultative anaerobic organisms and produce organic acids as their primary metabolites. While the homofermentative lactobacilli, such as L.acidophilus, produce only lactic acid, the heterofermentative lactobacilli, such as L.casei and L.reuteri, also produce acetic and phenyl lactic acids and gases. Although this feature contributes to the cariogenic mechanisms on one side since Lactobacilli co-participate as second colonizers in the mature supragingival dental plaque [50], they also limit the ability of *C. albicans* to switch to the hyphal phenotype, which is impaired in acidic environments [44]. Furthermore, some authors reported that some lactobacilli, such as L.*johnsonii*, can uptake nutrients from the oral environment, mainly sucrose, thus hampering fungal metabolism and leading to a lack of nutrients necessary for fungal growth [51].

3.1.2 Competition for Adhesion Sites and Protective Action on Mucosal Integrity (Biosurfactants)

Various Lactobacilli own a series of biosurfactant substances, partly cell-associated and partly cell-free. The first, as those present on *L. pentosus* surface, directly adhere, via receptor bindings, to keratinocytes and directly compete with fungal biofilm formation, thus excluding and displacing the yeasts from the mucosa surface [52–54]. The cell-free biosurfactants have anti-adhesive activity on planktonic *Candida* and disrupt the biofilm to which Candida cells adhere [54]. Other mechanisms through which Lactobacilli support the mucosal integrity lay on their exopolysaccharide (EPS) property to stimulate keratinocytes to produce mucins and not well-recognized pathways through which they allow the gut keratinocytes to increase the number of tight junctions, thus preserving the mucosa integrity against pathogens advance [55,56].

3.1.3 Production of Metabolites that Compromise the Integrity of the Fungal Wall and the Fungal Growth (Bacteriocins, Chitinases, Fatty Acids, Reuterin, Hydrogen Peroxide)

The primary Lactobacilli metabolites capable of disrupting the fungal wall are enzymes such as chitinase, which degrades the chitin of the yeast [57], and bacteriocins, such as plantaricin from *L.plantarum,* or pentocins from *L.pentosus*. Bacteriocins forms pores in the fungal cell membrane, thus altering extracellular-intracellular transports of electrolytes and small substances, favoring the increase of reactive oxygen species and leading to fungal cell death [58,59]. Similar mechanisms are shared by fatty acids, which alter the ergosterol synthesis and the fungal cell membrane permeability with fungicide effects [60].

All these changes, also supported by other Lactobacilli products, such as reuterin and hydrogen peroxide, increase the oxidative stress and the production and entering of reactive oxygens species (ROS), responsible for yeast apoptosis and necrosis [1,61].

The fungal growth and its metabolism based on anaerobic glucose fermentation are also interrupted by weak organic acids such as benzoic, acetic, and sorbic acids—still produced by Lactobacilli—which inhibit the production of ATP, necessary to the yeast, and leading to ATP and RNA depletion, with fungicide effects [62].

3.1.4 Stimulation of the Host Immune System Against Fungal Infections

Lactobacilli are also capable of interacting with the host through various mechanisms, mainly by defending and reinforcing the mucosal barrier, inducing the keratinocytes to produce and release antimicrobial proteins and peptides, such as protegrins and β-defensins, capable of disrupting the fungal cell membrane [63], downregulating the fungal-induced inflammation, and activating the immune responses [44,58]. In detail, different Lactobacilli species reduce the levels of different pro-inflammatory cytokines, mainly IL-2, IL-6, IL-17, and TNFα, and increase those of the anti-inflammatory ones, such as IL-10 [44]. The immune response activities promote macrophage migration and lymphocyte T, mainly CD4+, thus increasing the host's production of protective IgA, IgG, and IgM [44,64,65]. However, many of these activities have been reported *in vitro* and on animal models, while few reports exist on human clinical trials.

3.2 Bacilli

Similarly to Lactobacilli, which are closely related, Bacilli are rod-shaped, gram-positive bacteria accounting for over 200 species, partly aerobe obligate and partly facultative. Among them, *B. clausii* has been extensively studied for its beneficial effects on gastrointestinal disorders [66]. The main beneficial effects are due to its capability to colonize the gut mucosa, thus competing with pathogens bacteria, replacing them, and producing a series of antimicrobial bacteriocins, such as clausins and short-chain fatty acids, thus inactivating bacterial toxins and interfering with the fungal cell membrane, respectively. Furthermore, Bacilli also stimulate keratinocytes to produce mucins, preserve the mucosal barrier integrity, and modulate the host immune responses [66]. Probiotics preparations based on *B. clausii* strains are widely diffused in the market to treat diarrhea and gut dysbiosis. Furthermore, *B. clausii* can produce riboflavin (vitamin B2), an essential vitamin whose deficiencies in humans are associated with pathologic status sustaining and sustained by various diseases [67].

3.3 Bifidobacteria

Bifidobacteria are the most representative gram-positive bacteria of the gut microbiota, formerly known as *Lactobacillus bifidus*. Naturally found in fermented milk products, their name is due to the Y-shaped morphology ("bifid"). Primarily active in anaerobic habitats, their properties as similar to those reported for Lactobacilli and Bacilli. Furthermore, they also effectively reduce lactose intolerance, especially in infants [68]. However, poor literature investigated the antifungal effects of Bifidobacteria against orogastric candidiasis, reporting inhibition of fungal systemic dissemination and growth of *C. albicans* and decreased severity of gastric candidiasis in immunodeficient mice [69].

3.4 Saccharomycetes

Saccharomycetes belong to the kingdom of fungi and are widely present in food products such as fermented beverages, mainly beer and fruit juices. The principal investigated species as probiotics are *S. boulardii* and *S. cerevisiae*. From the same class but a different family, *Issatchenkia occidentalis* has also been applied in the food industry and is under study for its probiotic properties.

The probiotic mechanisms of these beneficial yeasts have been well recognized, particularly for *S. boulardii*, which is conventionally used to prevent and treat diarrhea, mainly antibiotic-associated, in adults and children [70]. The protective action of *S. boulardii* against bacterial infections results from the complementary effect of several mechanisms, mainly [71]:

- direct and indirect anti-toxin activities against *Clostridium difficile* and *Vibrio cholera*;
- prevention of the increase in intestinal permeability;
- direct effects on bacterial lipopolysaccharide (LPS) by its de-phosphorylating enzymes acting on enteropathogenic bacteria.
- Other effects have also been proven effective against *C. albicans* and other NAC, and they are shared both by *S. boulardii* and *S. cerevisiae* and reported as follows [71–75]:
- anti-inflammatory effect via the decrease of production of pro-inflammatory cytokines (TNFα, INFγ, IL-8, and IL-1) as well as the increase of anti-inflammatory cytokines (IL-4 and IL-10) in keratinocytes;
- prevention and inhibition of the hyphal switching and the fungal adhesion, colonization, and biofilm formation on different substrata;
- production of caprylic acid (*S. boulardii*) and other small bioactive molecules responsible for fungicidal effects;
- reduction of virulence gene expressions of *C. albicans* during infection (*S. cerevisiae*).

Some of these properties have also been referred to *I. occidentalis*, such as the inhibition of adhesion, co-aggregation, and biofilm formation, active both on *C. albicans* and NAC, and the production of an unidentified metabolite specifically inhibiting NAC virulence [76].

The main antifungal activities of the most investigated probiotics have been summarized in Table 1 (Ref. [44,51–56,58–61,66,71–75]).

4. Probiotics in Candidiasis: *in vitro* Studies and Clinical Experiences

The most promising *in vitro* effects on *C. albicans* from human volunteers were ascribed to L. *paracasei, L. rhamnosus, L. reuteri, L. delbrueckii ssp, and L. bulgaricus* [77–79]. Among them, *L. rhamnosus* and *L. reuteri* were proven effective also against vaginal isolates of *C. glabrata* strains [80].

Table 1. Antifungal activities of the most investigated probiotics.

Probiotic species	Antifungal compounds	Mechanisms of actions	Effects on Candida species	References
L.reuteri	lactic acid, acetic acid	the organic acids cross the hydrophobic fungal plasma membrane, dissociate within the cell and the charged anions induce cellular stress and disrupt membrane	creation of an environment hostile to the growth of Candida (acidification) with fungitoxic effects and growth inhibition	[44,61]
	1,3-propanediol as precursor of 3-hydroxypropionaldehyde (3-HPA, reuterin), phenyllactic acid	increase in the production of reactive oxygen species	growth inhibition	
	hydrogen peroxide	strong oxidative effect against fungal cells and damage of the molecular structure of essential proteins	cytotoxic effects, empowered in saliva by the presence of lactoperoxidase and thiocyanate	
L.pentosus, L.casei, L.rhamnosus, L.acidophilus, Bacillus subtilis, L.brevis, Lactobacillus spp	over 40 cell-associated biosurfactants (co-aggregation-promoting factor — Cpf, glycoproteins, glycolipids)	coaggregation with Candida spp. and competition in adhesion to keratinocytes	anti-adhesive and antibiofilm activities; antimicrobial activity proved on C.albicans, C.tropicalis, and C.krusei; protective action on mucosal integrity	[52,54]
	6 cell-free biosurfactants (glycoproteins or glycolipids)	coaggregation with Candida spp.; anti-adhesive activity on planktonic Candida and inhibition and disruption of the fungal biofilm	prevention of the adhesion of C. albicans on medical devices and reduction of biofilm formation	[54]
Lactobacilli spp	Exopolysaccharides (EPSs)	Stimulation of keratinocytes to produce mucins	reinforcement of the mucosal barrier to fungal infections	[55,56]
L.rhamnosus	fimbriae	mediation of adherence to mucus glycoproteins	prevention of the adhesion of C. albicans	[53]
Lactobacilli spp, L. plantarum, L.pentosus, B.clausii, S. boulardii, S. cerevisiae	bacteriocins (nisin, plantaricins, pentocins, clausins), chitinases, fatty acids, hydrogen peroxide, caprylic acid	Permeabilization of fungal cell membranes, through efflux of cations, leading to an efflux of small molecules and production of reactive species of oxygens	alteration of the integrity of the fungal cell membrane and the fungal growth thus leading to fungal death	[58–61,66,71–75]
L. johnsonii	uptake of nutrients from the environment	depletion of available nutrients to pathogens	Inhibition of the fungal growth	[51]
Lactobacilli spp, S. boulardii, S. cerevisiae	reduce the levels of pro-inflammatory cytokines, and increase those of the anti-inflammatory ones	downregulate the fungal-induced inflammation, increase the production of protective IgA, IgG and IgM, promote the macrophage and CD4+cells migration	stimulation of the host immune system against fungal infections	[51,71–75]

The first *in vivo* studies were on immunocompromised mice suffering from oral candidiasis. They are dated back to 1997 and 1998, when Wagner *et al*. [81,82] demonstrated the efficacy of three strains of lactobacilli *(L. acidophilus, L. reuteri, and L. casei GG)* and three *Bifidobacteria (B. animalis, B. lactis, and B. infantis)* in reducing the number of *C. albicans* cells, the incidence and severity of mucosal and systemic candidiasis, and contributing to significantly higher overall survival.

Two recent meta-analyses by Mundula *et al*. [17] and Hu *et al*. [83] summarized, in 2019, the evidence from the literature on the effects of probiotics on oral candidiasis in humans. In the total of 12 eligible papers, they found extreme heterogeneity in the studied populations, kinds of administrations, and outputs. Concerning population, a total number of 843 participants from ten countries was calculated; the most studied populations were made up of elders [84–89] and denture carriers [90–92], while few other works focused on children [93], young adults [94,95], or women [89]. The two systematic reviews reported a total of 16 strains of probiotics investigated, variously administered, alone or associated, during the weeks of study by lozenges, capsules, or via dairy products such as milk, cheese, or yogurt. Lactobacilli were the most recurrent probiotics used —mainly *L. reuteri, L. bulgaricus, L. casei, L. acidophilus*— followed by *Bifidobacteria (B. longum, B. breve)* and *Saccharomycetes (S. thermophilus),* while only one study reported the use of *Propionibacterium freudenreichii* combined with *L. rhamnosus.* Despite the heterogeneity of the studies, the meta-analysis revealed that probiotics showed a beneficial effect on reducing the levels of oral *Candida species*, with the most significant effects on denture wearers, particularly in those where the probiotics were applicated directly onto the denture surface, as reported by Ishikawa *et al*. [90] which used a capsule containing a mix of lyophilized *L. rhamnosus*, *L. acidophilus*, and *B. bifidum* maintained daily on the palatal surface of the maxillary denture. The authors reported that this administration dramatically reduced to 17% the number of dentures wearers with oral candidiasis at the end of treatment, compared with the placebo group, in which 92% of subjects were still Candida carriers.

Furthermore, other works have experienced these and other probiotics against human oral candidiasis. Kumar *et al*. [96] described a randomized control clinical trial on 150 children referred to a pediatric intensive care unit and undergone broad-spectrum antibiotics for at least two days. Of them, half were treated with a placebo and half with one sachet of a probiotic mix consisting of *L. acidophilus, L. rhamnosum, B. longum, B. bifidum, S. boulardii,* and S. *thermophilus* twice a day. The authors reported significantly less candiduria in test groups compared with the placebo, although candidemia was similar in both groups [96].

In 2919 Nirmala *et al*. [97] reported the encouraging results of *B. clausii* in a case-control study on subjects with recurrent aphtous stomatitis or oral candidiasis. They showed significant improvement in erythema, pain, burning sensation reduction, and decreased oral thrush within the fifth day of administration compared to the placebo group [97].

Another probiotic commercially available is *Streptococcus salivarius K12*, investigated in association with nystatin and whose efficacy was compared with a placebo receiving only nystatin in an RCT on 56 adult patients suffering oral candidiasis by Hu *et al*. [98]. The authors reported significantly higher percentages of complete eradication of Candida spp. in the group treated with *S. salivarius* K12 (over 90%) than in those in the placebo group (55%), with significantly higher remission of clinical symptoms and signs in a shorter time. No adverse effects were reported peculiar to the test group, concluding the safety of the probiotic used. A similar work by Li *et al*. [87] confirmed the potentiating effect of probiotics in nystatin-based treatment on an RCT on 65 subjects suffering oral candidiasis; in this case, the probiotic mix was made up of *B. longum, L. bulgaricus*, and *S. thermophilus* and the detection rate of Candida spp. after treatment, although decreased in both groups, was significantly lower in the probiotic+nystatin group (8%) than the nystatin group (35%).

Recently, probiotics have also been promising for treating oral candidiasis in patients who have undergone head & neck radiotherapy for oral cancer. Doppalapudi *et al*. [99] studied the effects of probiotics alone and in association with clotrimazole in 90 oncological patients after head and neck radiotherapy. In this case, the probiotic mix consisted of mouthwash with four probiotic strains (*L. acidophilus, L. rhamnosus, B. longum,* and *S. boulardii*) gargled three times daily for one month. Two other groups received clotrimazole alone or associated with the probiotic mix, respectively. At the endpoint, all three groups reported a reduction in mean Candida spp. count, despite it being significantly higher in the two groups using the probiotic mixture. The authors also identified the Candida species, revealing *C. albicans* overall decreased in all groups, *C. tropicalis* significantly decreased only in the probiotic group, and *C. glabrata* decreased in the probiotic group but persisted in the clotrimazole-treated one [99].

Other studies proposed some beneficial yeasts to treat oral candidiasis. The most studied and used one is *Saccharomyces boulardii*, whose safety and efficacy in humans have already been proven in re-establishing a healthy gut flora following diarrhea [100], thus favoring its introduction in the market and clinical practice. Concerning its application against candidiasis, in 2013, Demirel *et al*. [101] reported their encouraging experience with oral administration of five billion CFU per day of *S. boulardii* added to breast milk formula in 91 premature infants to prevent fungal infections, compared with the conventional use of

Table 2. Main studies reporting the use of probiotics in candidiasis.

First author, (year), [ref]	Type of study	Probiotics strains investigated	Aims	Type of Candidiasis	Candida species tested	Main outcome
Wagner (1997) [81]	*in vivo* study on immunocompromised mice	*L. acidophilus, L. reuteri, L. casei GG, Bifidobacterium animalis*	evaluation of the effects of probiotics on overall survival and inhibition of systemic dissemination of gastro-intestinal candidiasis	oropharyngeal candidiasis	*C. albicans*	the mice treated with each probiotic had a statistically significantly higher overall survival and lower incidence of systemic candidiasis; L. casei GG and B. animalis reduced the counts of C. albicans present in the alimentary tracts B. animalis reduced mucosal candidiasis incidence and severity
Wagner (1998) [82]	*in vivo* study on immunocompromised mice	*Bifidobacterium infantis, Bifidobacterium lactis*	evaluation of the comparative effects of two Bifidobacteria to increase the survival and reduce the systemic dissemination of gastro-intestinal candidiasis	oropharyngeal candidiasis	*C. albicans*	both probiotics guaranteed a statistically significantly higher overall survival and inhibited the incidence of systemic candidiasis
Sookkhee (2001) [77]	*in vitro* observational study on saliva from 130 healthy human volunteers	*L. paracasei, L.rhamnosus*	identification of endogenous salivary bacteria with antifungal activities in vitro	-	*C.albicans*	probiotic strains showed antifungal effects by inhibition of C.albicans growth
Petti (2001) [94]	randomized double-blind placebo-controlled trial on 42 healthy young adults	fruit yoghurt with and without probiotics (not specified)	assessment of any change in salivary counts for total viable flora, cariogenic bacteria, and Candida spp.	healthy young adults	*Candida spp.* and *cariogenic oral bacteria*	Candida spp. count was halved after daily yoghurt administration and the decrease was statistically higher in the test group
Hatakka (2007) [84]	randomized double-blind placebo-controlled study on 276 elderly people	*L. lactis,L.helveticus, L. rhamnosus GG (ATCC 53103), L. rhamnosus LC705, Propionibacterium freudenreichii ssp shermanii*	evaluation of the effects of cheese containing probiotic bacteria to reduce the prevalence of oral Candida species	Not specified	*C. albicans, C. glabrata, C. krusei, C. tropicalis.*	32% reduction of yeast prevalence in subjects treated with probiotic cheese; probiotic cheese reduced the risk of high yeast counts by 75% and the risk of hyposalivation by 56%
Mendonça (2012) [89]	clinical cohort study on 42 healthy elderly women	*L. casei, Bifidobacterium breve (formulation commercially available)*	evaluation of the probiotic influence on oral Candida prevalence and immunological response against Candida	Candida carriers without symptoms	*C. albicans, C. tropicalis, C.guilliermondii, C. glabrata, C. lipolytica, C. krusei, C. kefyr, C. parapsilosis*	the probiotic administration reported statistically significant reduction in Candida prevalence and counts; eradication of Candida spp. in 12% of women (all kind of strains); significant increase in anti-Candida IgA levels
Denkova (2013) [79]	*in vitro* on cultures	*L. acidophilus A2, L. acidophilus Ac, L.delbrueckii ssp. bulgaricus GB, B.bifidum sp. 4*	evaluation of antifungal activity in co-cultures		*C. albicans*	all probiotics restrained fungal growth, mainly L. acidophilus A2, and L. acidophilus Ac, due to their lactic and other organic acids accumulation over the time

Table 2. Continued.

First author, (year), [ref]	Type of study	Probiotics strains investigated	Aims	Type of Candidiasis	Candida species tested	Main outcome
Burton (2013) [93]	randomized double-blind placebo-controlled trial on 100 children	*Streptococcus salivarius M18*	evaluation of the change in plaque score and salivary levels of cariogenic bacteria and Candida species	dental caries-active children	*Candida spp.* and *cariogenic oral bacteria*	the salivary levels of Candida spp. did not differ substantially at the end of treatment from the baseline
Sutula (2013) [95]	clinical cohort study on 22 healthy volunteers	*L.casei strain Shirota (probiotic drink commercially available)*	evaluation of the effects on Candida spp. counts and the persistence of the probiotic strain in the oral cavity after administration	dentate healthy individuals	*C. albicans, C. parapsilosis, C.guilliermondii, C. krusei*	Significant but temporary and consumption-dependent presence of L. casei in saliva and plaque; Candida count was unaffected.
Kumar (2013) [96]	prospective double blind randomized controlled trial on 150 children	*L. acidophillus, L. rhamnosum, B.longum, B. bifidum, S. boulardi, S.thermophilus, fructo-oligosaccharides (sachets)*	evaluation of the efficacy of probiotics in the prevention of Candida colonization in feces, urine, and blood	PICU* neonates on broad spectrum antibiotics	*C. albicans, C.tropicalis*	37% reduction in prevalence of Candida colonization after two weeks of probiotic administration; Candiduria was significantly less common in the probiotic group than in the placebo group (17.3% vs. 37.3%); Prevalence of candidemia did not differ significantly in two groups
Demirel (2013) [101]	prospective, randomized comparative study on 180 newborns	*S. boulardii*	comparing the efficacy of orally administered probiotic versus nystatin in the prevention of fungal colonization and invasive fungal infections	very low birth weight preterm infants at risk for invasive fungal infections	*C.albicans*	S. boulardii supplementation was as effective as nystatin in reducing fungal colonization and invasive fungal infection; S.boulardii was significantly higher more effective in reducing the incidence of clinical sepsis and number of sepsis attacks: probiotic favorable effect on feeding intolerance
Li (2014) [87]	randomized controlled trial on 65 subjects	*B. longum, L. bulgaricus, S. thermophiles*	evaluation of the short-term efficacy and safety of probiotics added to conventional therapy (2% nystatin) in the treatment of oral candidiasis	clinically and microbiologically proven oral candidiasis	*Candida spp.*	both groups showed a significant reduction in pain level and lingual hyperemia. The detection rate of Candida spp. after treatment decreased in both groups and was significantly lower in the probiotic+nystatin group (8%) than the nystatin group (35%).
Kraft-Bodi (2015) [86]	double-blind randomized placebo-controlled study in 215 frail elderly patients	*L.reuteri* (DSM 17938 and ATCC PTA 5289) (oro-soluble lozenges)	camparing the effects of a daily intake of two probiotic lactobacilli on the prevalence and counts of oral Candida	not specified	not specified	statistically significant reduction in the prevalence of salivary and plaque high Candida counts in both the probiotic groups. Daily use of probiotic lozenges may reduce the prevalence of high oral Candida counts in frail elderly.

Table 2. Continued.

First author, (year), [ref]	Type of study	Probiotics strains investigated	Aims	Type of Candidiasis	Candida species tested	Main outcome
Ishikawa (2015) [90]	randomized double-blind placebo-controlled study on 59 elderly denture wearers	*L. rhamnosus HS111, L. acidophilus HS101, B. bifidum*	evaluation of the short-term effects of probiotics in reducing the levels of oral Candida	Candida spp. carriers with asymptomatic candidiasis	*C.albicans, C.guilliermondii, C. tropicalis, C. glabrata, C. dubliniensis,C. famata, C. parapsilosis.*	after the experimental period, the detection rate of Candida spp. was 92.0% in the placebo group and 16.7% in the probiotic group
Miyazima (2017) [91]	randomized double-blind placebo-controlled study on 60 denture wearers harboring oral Candida spp	*L.acidophilus NCFM* or *L. rhamnosus Lr-32*	evaluation of the effects of consumption of two experimental probiotic-cheeses on the oral fungal colonization in denture wearers	Candida spp. carriers with asymptomatic candidiasis	*C.albicans, C. glabrata, C. dubliniensis, C. tropicalis, C. krusei, C. famata, C. kefyr*	the mean levels of Candida spp. were significantly reduced in both group treated with probiotic cheese, strongly in that enriched with L.acidophilus
Jørgensen (2017) [78]	*in vitro* on fungal cultures	*L.reuteri* (strains DSM 17938 and PTA 5289)	evaluation of the ability to co-aggregate with Candida spp. and to inhibit the growth of the yeasts assessed	-	*C.albicans, C. glabrata, C. krusei, C. tropicalis, C. dubliniensis, C. parapsilosis*	both probiotic strains almost completely inhibited the growth of C. albicans and C. parapsilosis, but did not affect C. krusei; L.reuteri PTA 5289 showed statistically significant higher fungal co-aggregation and growth inhibition; C. krusei resisted the acids produced by the lactobacilli
Rane (2018) [92]	cohort study on 60 completely edentulous denture wearers	not specified	Evaluation of the effects of probiotics on the prevalence of oral Candida spp. on oral mucosa and denture surfaces	healthy Candida spp. carriers	*C.albicans, C. glabrata, C. tropicalis*	at the end of the treatment, Candida colonies significantly decreased both on mucosa and mainly on denture surfaces
Lopez-Jornet (2018) [88]	clinical cohort study on 27 elderly patients	*L. reuteri* DSM 17938 and ATCC PTA 5289 (tablets)	evaluation of the short-term clinical efficacy against Candida levels and the safety of probiotics	14 subjects with normal Candida counts and 13 with elevated count	Not specified	improvement in burning sensation and saliva flow after treatment; the Candida count did not statistically differ at the beginning and at the end of treatment
Keller (2018) [85]	double-blinded randomized placebo-controlled intervention study on 22 patients with oral lichen planus and oral candidiasis	*L.reuteri (DSM 17938 and ATCC PTA 5289) (oro-soluble lozenges)*	evaluation of the effects of probiotics in reducing the recurrences of oral candidiasis and the Candida count/carriage	Oral candidiasis superinfection on oral lichen planus	Not specified	probiotic intervention did not reduce recurrencies of oral candidiasis or Candida count/carriage

Table 2. Continued.

First author, (year), [ref]	Type of study	Probiotics strains investigated	Aims	Type of Candidiasis	Candida species tested	Main outcome
Nirmala (2019) [97]	double blind randomized controlled trial on 80 young adults	*Bacillus Clausii, (oral application of probiotic)*	assessment of the efficacy of local application of oral probiotic in treating recurrent aphthous ulcers and oral candidiasis	adults suffering recurrent aphthous ulcers or oral candidiasis	*Candida spp*	the test group reported significant improvement in erythema, pain reduction, decreased oral thrush, and burning sensation in the mouth
Hu (2019) [98]	randomized double-blind placebo-controlled clinical trial on 56 patients	*Streptococcus salivarius K12*	evaluation of the efficacy and safety of Streptococcus salivarius K12+ nystatin vs nystatin alone (lozenges) as an adjuvant in treating oral candidiasis	oral candidiasis	*C. albicans, C. tropicalis, C glabrata, C. krusei*	the probiotic+nystatin group reported significantly higher remission of clinical symptoms and signs in a shorter time, where fungal counts were significantly lower; S.salivarius K12 exhibited potential efficacy and safety as an adjuvant in treating oral candidiasis
Roselletti (2019) [102]	randomized double-blinded placebo-controlled clinical trial on animal models	*S. cerevisiae CNCM I-3856 (live or inactivated cells), L. rhamnosus GG*	to investigate the effectiveness of probiotic strains against oropharyngeal candidiasis	mice with oropharyngeal candidiasis	*C.albicans*	both live and inactivated S. cerevisiae significantly decreased the C. albicans load and counts in the oral cavity; no effect was observed for L. rhamnosus; both live and inactivated S. cerevisiae had beneficial effects in the stomach; only live S. cerevisiae had beneficial effects in the duodenum
Doppalapudi (2020) [99]	randomized clinical trial on 90 patients	*L. acidophilus, L. rhamnosus, B.longum, S. boulardii (sachet commercially available*	assessment of the effect of probiotic bacteria alone or in association with clotrimazole, vs placebo, on oral Candida counts	Candida carriers oncological patients who completed head- and neck-radiotherapy	*C.albicans, C.glabrata, C.tropicalis, C.parapsilosis, C. krusei*	after intervention, the probiotic formulation, both alone or in combination with clotrimazole, was effective in reducing oral Candida spp. counts; in the "probiotic alone" group, C.albicans, C.glabrata and C.tropicalis were the most responsive species, while C.glabrata was the less responsive one

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nystatin in a group control of 90. The authors reported similar prophylactic efficacy in both groups in reducing fungal skin colonization and invasive infections and probiotic milk had higher effects than nystatin to reduce the incidence of clinical sepsis, similarly to what was reported by Kumar *et al*. [96] and described above.

Apart from *S. boulardii*, other yeasts currently under study are *S. cerevisiae*, usually found in beers, wine, and bread, and *I. occidentalis,* in fermented fruit and juices. The antifungal properties of *S. cerevisiae* have been proven both in mice with oropharyngeal candidiasis [102] and with vaginal candidiasis [103], where, after local administration, it was able to decrease the levels of *C. albicans* and resolve local inflammation, parallel to an increase in neutrophilic antifungal activity.

The main mechanisms considered by the authors were the co-aggregation between Candida species and *S. cerevisiae*, which are responsible for inhibiting Candida adhesion to the mucosal surfaces and protecting the keratinocytes from Candida damage.

The probiotic properties of *S. cerevisiae* and *I. occidentalis* have been widely explained *in vitro* and *in vivo*, in cells and animal models, studies [73–75], which proposed them as alternative probiotics against candidiasis. For both of them, the authors reported a series of anticandidal properties against a series of NAC such as *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, and multidrug-resistant *C. auris,* increasingly responsible for nosocomial infections.

Furthermore, the use of *S. cerevisiae* was also suggested by Shibasaki *et al*. [103,104] as the basis for vaccines against Candida spp. with engineered *S. cerevisiae* and *L. casei* expressing a candida antigen — Eno1p antigen— on their surfaces. The vaccines, administered orally to mice, were shown to protect 60% of mice against a lethal dose of C. albicans cells and significantly increase their overall survival compared with not vaccinated mice. However, it must be considered that in some cases, such as oncologic or HIV/AIDS patients, *S. cerevisiae* is responsible for fungal-resistant infections [105,106].

The most promising studies reporting the use of probiotics in candidiasis have been chronologically reported in Table 2 (Ref. [77–79,81,82,84–99,101,102].

5. Discussion and Conclusions

The beneficial role of probiotics as a therapeutic alternative in fungal infections is becoming increasingly consistent and evident. Although the literature reports only a few works concerning their application for oral candidiasis in humans, the potential benefit of this alternative treatment is intuitable. Some strains were proven to reduce the Candida loads and counts in the oral cavity and dentures and significantly reduce hyposalivation and symptoms linked with the fungal infection. Furthermore, when associated with conventional antifungal drugs, some probiotics reduced the therapies' duration by accelerating the responsiveness, significantly preventing candiduria and systemic candidiasis dissemination and reducing the risk of clinical sepsis in at-risk subjects, such as oncologic patients and preterm low birth weight neonates under antibiotic therapy. The need for shorter and/or lesser dosages of antifungals could fight the risk of resistance and the side effects of the drugs.

However, the smallness and heterogeneity of the studies conducted on humans do not allow us to establish, to date, defined and definitive therapeutic schemes. Furthermore, other significant limitations have emerged from the analysis of the literature. Both commercially available probiotics and experimental mixtures reported in the studies are multi-strain preparations, and their specificity has not been fully assessed *in vivo*. Furthermore, none of the studies considered the viability and measured the durability of the strains in the oral cavity, except for one work. Considering that some probiotics are not commonly resindent of the oral cavity, it is hard to hypotesize their effective establishment in the oral microenvironment and capability to compete proficiently with authologus species, thus posing far from defining a proper "personalized" approach.

Considering the highly variable composition of the oral microbiome among individuals by sex, age, diet habits, and co-morbidities, it is reasonable to hypothesize that some probiotics work better in some subjects than others.

Indeed, in order to concretize a proper personalized probiotic therapy, a greater understanding and knowledge of the oral microbiota and the biological and metabolic interconnections between its various constituents and the interactions with the host is desirable. This progress will allow the possibility of specific and tailored applications as a concrete, safe and feasible alternative to the use and abuse of conventional antifungals, towards which are registered forms of drug resistance more and more numerous.

The hope is that a more significant number of omics and clinical studies will allow us to resolve the doubts and unknowns that still exist. Based on these perspectives and considering the individual variability of the oral microbiota, it is reasonable to move towards personalized medicine and dentistry that meet each individual's specific needs by proposing a specific probiotic mix.

Author Contributions

MC was responsible for conceptualization, methodology, analysis, data curation, writing the original draft and writing, reviewing & editing the final version.

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Conflict of Interest

The author declares no conflict of interest.

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