Probiotics as an alternative antimicrobial therapy: Current reality and future directions

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ABSTRACT

Probiotics are defined as microorganisms that live in symbiosis with the human host. When ingested in adequate quantities, probiotics may modulate biological functions, with health benefits. Different biological properties have been reported for probiotics, including antimicrobial activity. However, there are few studies investigating the use of probiotics as candidates for alternative antimicrobial therapy or as a source of new antibiotics. Thus, in this review we provide a general approach to the current situation of probiotic antimicrobial research and point out future directions in the field. Despite the promising benefits of probiotics on intestinal health, there remains no consensus or standardization on the development of delivery systems and on the application of probiotic formulations for antimicrobial therapy. Thus, further bioguided studies and clinical trials are needed to address the existing gaps in the knowledge. Future research should focus on the isolation, doses, clinical efficacy, safety and mechanisms of action of probiotics in humans.

1. Introduction

Immediately after birth, the human body is colonized by different microorganisms, such as archaea, bacteria, fungi, viruses and micro-eukaryotes (Aagaard et al., 2014). Over time, colonization occurs so intensely that the human microbiome of an adult individual contains more bacterial cells than human cells (Sender, Fuchs, & Milo, 2016). Different types of microorganisms can cause disease in humans and some of which have a high fatality rate (Peterson et al., 2009). For many years, scientific research has focused on understanding pathogenic bacteria and finding ways to preventing and treating human diseases. Conversely, some bacterial species may bring benefits to the host through a symbiotic relationship. These microorganisms are generally named probiotics (Fijan, 2014).

Probiotics are living microorganisms that provide health benefits when ingested in adequate amounts (FAO/WHO, 2001). Most probiotic bacteria are Gram-positive, and their main functions are related to modulation and maintenance of the intestinal tract health (e.g., Lactobacillus and Bifidobacterium) (Marco, Pavan, & Kleerebezem, 2006).

The probiotics that colonize the human host are most numerous in the intestines. The commensal intestinal microbiome contributes to increased resistance against infections, host immune system differentiation, and synthesis of nutrients (Ubeda & Pamer, 2012).

There is evidence that probiotics may act in the treatment and prevention of infectious diseases (Yang et al., 2019). Currently, infectious diseases are commonly managed with the administration of antibiotics. However, an irrational use of antibiotics may cause consequences at the patient level, such as drug-specific adverse effects, and at the public health level, such as selection of multidrug-resistant bacteria (Yang et al., 2019). Thus, the search for new alternatives in antimicrobial therapy is much needed, with a special interest in natural product-based therapies (Silva et al., 2019).

Clinical trials have shown that probiotics are effective against a wide range of pathological conditions, such as constipation, diarrhea, polycystic ovary syndrome, ulcerative colitis, stress and anxiety.
inflammatory bowel disease, breast cancer and diabetes (Kechagia et al., 2013).

Despite the proven biological properties of probiotics, such as antimicrobial activity, research in this area is still incipient and needs further discussion. Thus, this review provides a general approach to the current situation of probiotic antimicrobial research and points out future directions in the field.

2. Probiotics

Ancient civilizations, such as Greeks and Romans, developed recipes for fermented milk, and the Bible mentions sour milk a few times. Thus, the beneficial effects of lactic acid fermentation on human health have been long discussed (Hosono, 1992).

Probiotics are living microorganisms (yeast or bacteria) that provide beneficial effects while colonizing the host. Lactic acid bacteria species (Lactococcus, Lactobacillus, Streptococcus and Enterococcus) and Bifidobacterium (Doron & Snydman, 2015; Prado & de Lindner, 2015; Soccol et al., 2015) are among the best-known probiotics. These microorganisms have characteristics that give them the ability to withstand adverse conditions in the host organism, such as enzymatic action and acidity. They can colonize the host and contribute to health by regulating the microbiome and performing biological functions (de Melo Pereira et al., 2018).

There is mounting evidence on the biological efficacy of probiotics; however, the indication of these microorganisms for clinical use should be seen with caution (de Melo Pereira et al., 2018). In 2002, the United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO) published the “Guidelines for the Evaluation of Probiotics in Food.” These guidelines established safety and efficacy standards for probiotics, systematizing their discovery and selection (Araya et al., 2002). Thus, the FAO/WHO guidelines suggest different criteria that should be evaluated for the selection of probiotics, namely: resistance to unfavorable conditions in the human body, ability to adhere to epithelial tissues, antimicrobial activity, and safety for use (de Melo Pereira et al., 2018). The safety of a probiotic strain is defined by its origin and lack of association with pathogenic cultures, in addition to its antibiotic resistance profile (Markowiak Slizewska, 2017a,b).

Lactobacillus is considered the oldest documented probiotic. It is a genus with Gram-positive bacteria of the LAB group. These rod-shaped bacteria comprise about 183 known species and are commonly applied in different industrial food processes (König & Fröhlich, 2009).

The main biological mechanisms of action of probiotics include increased epithelial barrier, increased adhesion to the intestinal mucosa and inhibition of microbial adhesion and competitive exclusion of pathogenic microorganisms, production of antimicrobial substances and immune system modulation (Bermudez-Brito et al., 2012). Fig. 1 shows a schematic representation of how these mechanisms occur in the intestinal mucosa.

Microorganisms of the LAB group produce lactic acid from different carbon sources, such as simple carbohydrates (Carr, Chill, & Maida, 2002). In addition, they eliminate secondary metabolites (e.g., bacteriocins, exopolysaccharides and enzymes) that inhibit the growth of other microorganisms. These factors are related to the different antimicrobial mechanisms of action of probiotics (Leroy & De Vuyst, 2004, de Melo Pereira et al., 2018). These mechanisms are well understood in improving bowel function, as shown in Fig. 1. However, the literature is still incipient regarding the potential of probiotics for alternative antimicrobial therapy against infectious diseases.

3. Methods for determining the antimicrobial activity of probiotics against other microorganisms

There are different in vitro methods for determining the antimicrobial activity of a substance. In the case of probiotics, it is possible to determine a direct antagonism between a probiotic culture and that of a pathogenic strain or to determine the antimicrobial activity of a probiotic extract (planktonic cells) (Fijan, 2016). When the purpose of the analysis is merely to discover the antagonism of one microorganism in relation to another, then microbial antagonism assays on solid media are most appropriate (Tagg, Dajani, & Wannmaker, 1976; Balouiri, Sadiki, & Ibnsouda, 2016). This approach involves the detection of growth inhibition of an indicator strain caused by the test culture. In this section, we make a critical analysis of the main methods currently available for in vitro evaluation of the antimicrobial activity of probiotics.

The Agar Spot Test was described by several authors (Tagg et al., 1976; Tharmaraj & Shah, 2009; Choi & Chang, 2015; Macaluso et al., 2016), with several modifications over time. We describe here the variation of this method that is mostly indicated to determine the antimicrobial activity of probiotics. There are two variations of this method that are commonly used, namely: simultaneous (or direct) and
deflected antagonism. In the direct assay, the test and indicator cultures are grown simultaneously, and the demonstration of antagonism depends on the release of a diffusible inhibitor at the beginning of the test culture growth (Tagg et al., 1976). In deferred antagonism, the probiotic microorganism under test is grown on agar media for a certain period and then inactivated; next, an overlap of the indicator strain is placed on the surface of the on the molten agar. This method is considered more sensitive and allows an independent variation of time and incubation conditions of test and indicator cultures (Tagg et al., 1976). After incubation, the antimicrobial activity is expressed either as an inhibition zone (mm) or as arbitrary units (AU/ml).

The Agar Well Diffusion assay can be used to determine the antagonistic effects of cell-free supernatants. Different nutrients, selective or differential media, are prepared. The plates are inoculated with the indicator microorganism. Subsequently, 6-mm or 7-mm wells are prepared in each plate. The supernatant of the probiotic microorganism is centrifuged and diluted in aliquots at different concentrations and then pipetted into the wells. After incubation, the antimicrobial activity is expressed as an inhibition zone or as arbitrary units (AU/ml) (Tagg et al., 1976; Parente, Brienza, Moles, & Ricciardi, 1995). We do not recommend using the disk diffusion method for this purpose because of standardization issues due to variations between the viscosity of the test substance and the physical differences of the discs (Hoelzer et al., 2011; Balouiri et al., 2016).

A study tested 104 strains of Lactobacillus acidophilus to compare broth microdilution, disc diffusion and Etest methods in determining the antimicrobial activity of probiotics. Except for some specific agent-related effects, there was a good agreement between Minimum Inhibitory Concentration (MIC) values in the broth microdilution method and the Etest. Another study demonstrated a higher capacity of cell-free supernatants of Lactobacillus plantarum strains against pathogenic bacteria in liquid medium than on agar plates (Mayrhofer et al., 2008). However, given the importance of obtaining a MIC value for determining possible dosages in in vivo tests (Turnidge, 1990; Mouton et al., 2018), we recommend the broth microdilution method when the objective of the analysis is to screen biomolecules for their potential antimicrobial drug.

Microdilution is one of the simplest and most reproducible methods for antimicrobial susceptibility screening. The procedure involves the preparation of 1:2 dilutions of the antimicrobial agent (cell-free supernatant), (e.g., 32, 64, 138, 256, 512 µg/mL) in a liquid growth medium dispensed into a 96-well microplate. Subsequently, each well is inoculated with a standard inoculum of the pathogenic strain (0.5 McFarland) and the plate is incubated after mixing under conditions appropriate for each microorganism. The broth microdilution method provides the MIC value, which is the lowest concentration of the antimicrobial agent that completely inhibits microbial growth (Balouiri et al., 2016). For details on broth microdilution testing and its specific conditions for each microorganism, we suggest consulting the Clinical Laboratory Standards Institute (CLSI) guidelines.

The antimicrobial activity proven by agar susceptibility or broth microdilution methods unfortunately does not characterize a probiotic microorganism as promising, as there are other factors to consider. In the microbiological viewpoint, an extremely important factor is the ability of microorganisms to develop as biofilms (Flemming et al., 2016). Bacterial biofilms are formed by communities embedded in a self-produced matrix of extracellular polymeric substances. Organized as a biofilm, microorganisms exhibit different living conditions than when in planktonic growth. Biofilms also serve as a physical barrier and exhibit a genotype that provides increased virulence, which makes them up to 1000 times more resistant to antimicrobials than planktonic bacteria (Donlan & Costerton, 2002; Marsh, 2004; Fleming et al., 2016).

A standardized assay to assess the activity of antibiofilm agents has not been established yet. However, there are different methods available for studying biofilms as well as for evaluating the antibiofilm activity of a substance. Several approaches have been standardized for this purpose, such as modified Robbins device, Calgary biofilm device, disk reactor, Centers for Disease Control (CDC) biofilm reactor, perfused biofilm fermenter, and bladder model (Kirmusaoğlu, 2019). The analysis of biofilms involves many techniques ranging from older established methods – such as counting of bacterial colonies – to more modern techniques – such as fluorescent labeling of biofilms in combination with mathematical predictive modeling, such as COMSTAT (Wilson et al., 2017). The studies screening for the antibiofilm activity of probiotics are even more limited than those testing planktonic cultures. However, some studies have evaluated this biological activity of probiotics by different methods and with different ways of analyzing the results (Missaoui, Saidane, Mzoughi, & Minervini, 2019; Manna, Ghosh, & Mandal, 2019; Hager et al., 2019; Mahdhi et al., 2018; Abdelhamid, Essam, & Hazaa, 2018; Aarti et al., 2018; Cui, Yan et al., 2018; Cui, Shi et al., 2018). There is no consensus on what the most cost-effective method is to determine the antibiofilm activity of probiotic microorganisms.

Another challenge for the alternative antimicrobial therapy or any other therapy is that in vitro results can be reproducible in in vivo models and, subsequently, in the human body. In vivo testing should be very well designed to avoid bias. To establish the efficacy of the probiotic product, randomized, double-blind, placebo-controlled clinical trials should be performed (Fijan, 2016). To determine if a probiotic can prevent or treat a specific pathogen infection, two types of study can be performed: a preventive study (clinical study to check if exposure to that pathogen is prevented after probiotic use) or an interventional study (prior exposure to the pathogen and subsequent treatment with the probiotic or its supernatant) (Fijan, 2016). In many cases, animal model results are not reproducible in humans. To prevent this problem, it is possible to use alternative methods, such as 3D cell cultures and human tissues.

Although clinical trials in humans are considered mandatory for establishing the health benefits of probiotics, a few strains that showed positive results have been employed after legal authorities were convinced about these health claims. This may dramatically impact the validity of workflows currently used to characterize probiotics (Papadimitriou et al., 2015). Thus, in order to optimize and standardize the selection and use of probiotics for antimicrobial therapy, we recommend conducting research in the form of a bioguided study. Bioguided studies aim to monitor the biological activity of interest, increasing the chances of isolating a compound with high biological potential; this type of study has been indicated for the discovery and establishment of new therapies (Pieters & Vlietinck, 2005; Porte et al., 2014). Fig. 2 summarizes our proposed bioguided study for monitoring the antimicrobial properties of probiotic microorganisms.

4. Antimicrobial activity of probiotics against human pathogens

According to the United Nations Food and Agriculture Organization and the World Health Organization, probiotics are living microorganisms that confer a benefit to the health of the host when administered in adequate amounts (FAO/WHO, 2019).

Currently, the main probiotic microorganisms used by humans are Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, L. acidophilus, L. casei Shirata, L. paracasei, L. reuteri, L. johnsonii, L. plantarum e L. rhamnosus L. reuteri, L. rhamnosus, L. paracasei, Bifidobacterium bifidum, B. infantis, B. lactis, Saccharomyces boulardii, and Propionibacterium freudenreichii (Morais & Jacob, 2006; Lesbros-Pantoflickova, Corthesy-Theulaz, & Blum, 2007; Reddy & Narendara, 2010; Sikorska & Smoragiewicz, 2013; Markowiak Sliżewska, 2017a,b). In recent years, several studies have revealed benefits in the administration of probiotics, ranging from direct inhibition of pathogenic microorganisms to improvements in host immune system functions (Sajedinejad et al., 2017; Lopes, Moreira et al., 2017; Rossoni et al., 2017; Markowiak Sliżewska, 2017a,b; Goderska, Agudo Pena, &
Table 1 shows the main probiotic microorganisms with antimicrobial activity. Adapted from (Marco et al., 2006; Lesbros-Pantoflickova et al., 2007; Reddy & Narendera, 2010; Sikorska & Smoragiewicz, 2013; Ouwehand et al., 2016; Markowiak Slizewska, 2017a,b; Wonget al., 2019).

<table>
<thead>
<tr>
<th>Lactobacillus</th>
<th>Bifidobacterium</th>
<th>Other bacteria</th>
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<tbody>
<tr>
<td>L. acidophilus</td>
<td>B. bifidum</td>
<td>Saccharomyces boulardii</td>
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<tr>
<td>L. amylovorus</td>
<td>B. infantis</td>
<td>Propionibacterium freudenreichii</td>
</tr>
<tr>
<td>L. casei</td>
<td>B. lactis</td>
<td>Enterococcus faecalis</td>
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<tr>
<td>L. crispatus</td>
<td>B. adolescentis</td>
<td>Enterococcus faecium</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>B. animalis subsp. lactis</td>
<td>Lactobacillus lactis</td>
</tr>
<tr>
<td>L. casei Shirota</td>
<td>B. longum R0175</td>
<td>Leuconostoc mesenteroides</td>
</tr>
<tr>
<td>L. paracasei</td>
<td>B. breve</td>
<td>Pediococcus acidilactici</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td></td>
<td>Sporolactobacillus inulinus</td>
</tr>
<tr>
<td>L. reuteri</td>
<td></td>
<td>Streptococcus thermophilus</td>
</tr>
<tr>
<td>L. johnsonii</td>
<td></td>
<td>Escherichia coli</td>
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<tr>
<td>L. helveticus R0052</td>
<td></td>
<td>Saccharomyces cerevisiae var. boulard</td>
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<tr>
<td>L. fermentum</td>
<td></td>
<td>Bacillus coagulans</td>
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</table>

Fig. 2. Proposed bioguided study for monitoring the antimicrobial activity of probiotic microorganisms. (A) Probiotic microorganism isolated from any source. (B) Biomonitoring (B1 - determination of in vitro antimicrobial activity and B2 - probiotic resistance to hostile conditions. Upon confirming promising results in Biomonitoring 1, start Biomonitoring 2 (C), which is the in vivo analysis of antimicrobial properties. After obtaining promising results in an animal model, move forward to Biomonitoring 3 (D) to evaluate biological properties and safety in alternative models of 3D cell cultures and human tissues. At this point, two approaches are possible, namely: clinical trials in humans (E) to determine an alternative antimicrobial therapy, or the analysis and isolation of probiotic-derived molecules (F) to develop a new antibiotic (monodrug). After detection of supernatant components of the antibiotics and their isolation, these molecules should again pass through the three biomonitoring phases (F, G, H and I) added of toxicity assays. Finally, the most promising molecule is submitted to human clinical testing for the development of the new antibiotic.

The use of probiotics is intended to support the health of the host. The literature shows a large number of studies using probiotics, but most of them explain only how probiotics can maintain the intestinal health of the host. The mechanisms of action of probiotics are various, such as the production of inhibitory substances, such as bacteriocins and hydrogen peroxide, which inhibit Gram negative and Gram positive pathogenic bacteria; blockage of adhesion sites; competition for nutrients; among others (Kanmani et al., 2013; Neal-McKinney et al., 2012; Sikorska & Smoragiewicz, 2013; Markowiak Slizewska, 2017a,b; Moraes, Costa, Segundo, & Peruzzo, 2019). Probiotics participate in immune response modulation in several ways, namely: by increasing nonspecific phagocytic activity through macrophage activation (Jain et al., 2008) and altering the release of pro and anti-inflammatory cytokines (Dong, Rowland, & Yaspoob, 2012; Zhao et al., 2012; Ganguli et al., 2013; Plaza-Díaz et al., 2017).

Several probiotic species are widely used in research (Villena et al., 2018; Moraes, Costa, Segundo, & Peruzzo, 2019). The use of probiotics has intensified, but the vast majority of studies relate them to intestinal health (Underwood, 2019).
Table 2
In vivo and ex vivo studies reporting the antimicrobial activity of probiotics.

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Objective</th>
<th>Conclusion</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Lactobacillus casei</td>
<td>In vivo study, comparing the therapeutic effectiveness of the probiotic L. casei alone and together with antioxidant drugs against giardiasis in a murine model.</td>
<td>Oral administration of the probiotic L. casei associated with albendazole reduced Giardia infection, as evidenced by the normal recovered intestinal morphology.</td>
<td>Shukla et al., 2013</td>
</tr>
<tr>
<td>Lactobacillus rhamnosus HS111, Lactobacillus acidophilus HS101 and Bifidobacterium bifidum</td>
<td>Randomized double-blind study with 59 denture wearers who had Candida spp. in the oral cavity</td>
<td>Decrease in Candida spp. in individuals who used the probiotic formulation.</td>
<td>Ishikawa et al., 2015</td>
</tr>
<tr>
<td>Bifidobacterium animalis subsp. Lactis</td>
<td>Randomized controlled trial with 51 patients using yogurt for four weeks supplemented with B. animalis for periodontal health</td>
<td>The use of yogurt supplemented with B. animalis can have a positive effect against the accumulation of bacterial plaque and gingival inflammatory parameters.</td>
<td>Eren, Laleman, Yalnizoglu, Kuru, &amp; Teughels, 2017</td>
</tr>
<tr>
<td>Lactobacillus salivarius</td>
<td>Randomized double-blind study with development of an experimental mouthwash for the treatment of periodontitis.</td>
<td>The results suggest that the mouthwash containing probiotics was healthy for daily use as an alternative to maintain dental and periodontal health.</td>
<td>Sajedinejad et al., 2017</td>
</tr>
<tr>
<td>Lactobacillus paracasei</td>
<td>In vivo study. Lactobacillus paracasei was used to treat Candida albicans infection in Galleria mellonella larvae. The number of hemocytes and gene expression of antifungal peptides were further assessed.</td>
<td>L. paracasei was able to modulate the immune system of G. mellonella and protect against candidiasis.</td>
<td>Rossoni et al., 2017</td>
</tr>
<tr>
<td>Lactobacillus reuteri DSM 17938</td>
<td>Use of L. reuteri to ameliorate inflammation in an ex vivo skin model, and in vitro antimicrobial activity against skin pathogens.</td>
<td>The probiotic decreased the inflammatory process and presented antimicrobial action against S. aureus, S. pyogenes, Cutibacterium acne, and P. aeruginosa.</td>
<td>Khalamadze et al., 2019</td>
</tr>
</tbody>
</table>

2016; Yang et al., 2014; Yun, Oh, & Griffiths, 2014). Among these benefits are antimutagenic (Yu & Li, 2016; So, Wan, & El-Nezami, 2017), anticarcinogenic (Wollowski, Reckhemmer, & Pool-Zobel, 2001; Casas-Solis et al., 2019; Devaraj et al., 2019) and antidiarrheal properties (Liu et al., 2017; Devaraj et al., 2019), immune system stimulation (Casas-Solis et al., 2019), prevention of atopic dermatitis (Rather et al., 2016; Huang et al., 2017; Lise, Mayer, & Silveira, 2018) and reduced blood cholesterol (Shimizu et al., 2015; Nath et al., 2018). Thus, the use of probiotics has been considered a promising strategy for the prevention and control of various infectious diseases. Tahmourespour, Salehi, Kermanshahi, & Eslami, 2011; Ishikawa et al., 2015; Matsubara et al., 2016; Eren, Laleman, Yalnizoglu, Kuru, & Teughels, 2017; Sajedinejad et al., 2017; Lopes et al., 2017; Rossoni et al., 2017; Goderska, Agudo Pena, & Alarcon, 2018; Moraes, Costa, Segundo, & Peruzzo, 2019). Table 2 shows in vivo/ex vivo studies that validated the antimicrobial activity of probiotics.

There are a multitude of probiotic formulations that are supposed to benefit human health, including immunostimulatory effects or interbacterial competition between beneficial and pathogenic bacteria (Piewngam et al., 2019). In this context, the use of probiotics has been considered a promising strategy for the prevention and control of various infectious diseases. A study performed by (Lahtinen et al., 2007) showed that three of 38 Bifidobacterium strains were able to inhibit the growth of Staphylococcus aureus, which is commonly found in systemic and peri-implant infections. Lazarenko (2012) investigated the antibacterial activity of several probiotic Lactobacilli strains. The authors reported that Bifidobacterium bifidum (B. bifidum) was mostly effective against S. aureus in an intravaginal infection model in mice, with a significant reduction in the number of S. aureus cells from vaginal smears. B. bifidum showed the best anti-staphylococcal activity when compared to other probiotic strains of different genera.

Piewngam et al. (2018) identified an inverse correlation between human colonization with Bacillus species and S. aureus. The authors further discovered a primary mechanism by which Bacillus species can kill S. aureus through the inhibition of quorum sensing. Fengycins are a specific classes of lipopeptides secreted by Bacillus species – identified by chromatography and mass spectrometry – that also present anti-fungal activity (Chung & Raffatellu, 2019). Lee et al. (2019) studied the probiotic Pediococcus acidilactici HW01 against Pseudomonas aeruginosa (P. aeruginosa) and observed decreased motility of P. aeruginosa as well as decreased production of pyocyanin, decreased production of proteases and rhamnolipid, and decreased biofilm formation on the surface of stainless steel. Another study conducted by Moraes, Costa, Segundo, and Peruzzo (2019) showed that Lactobacillus brevis and Bifidobacterium bifidum were effective against S. aureus biofilms grown on titanium discs. The results showed reduction of S. aureus growth on titanium discs when both probiotics were used, but the greatest inhibitory effect on biofilm formation was observed for L. brevis strains.

Other study performed by Sikorska and Smoragiewicz (2013) demonstrated that the probiotics Lactobacillus reuteri, L. rhamnosus GG, Propionibacterium freudenreichii, P. acnes, L. paracasei, L. casei, L. plantarum, L. bulgaricus, and L. fermentus inhibited methicillin-resistant S. aureus (MRSA) biofilm formation, possibly by competition and production of acids and/or bacteriocin inhibitors.

A review performed by Goderska et al. (2018) showed that Helicobacter pylori (H. pylori) has been regarded as a difficult-to-treat infection mainly because of acquired resistance to commonly used antibiotics. There is a growing interest in using probiotics in combination with antibiotic regimes to eradicate H. pylori. Probiotics have been proven to be useful in the treatment of several intestinal diseases such as diarrhea, in addition to the benefits of probiotic bacteria to the intestines; some beneficial effects on the stomach have also been reported, including anti-Helicobacter pylori activity (Aiba et al., 2017). The benefits of probiotic therapy in H. pylori cases are decreased microbial load and improved host tolerability. Several studies have shown favorable effects of different probiotics against H. pylori by strengthening the mucosal barrier, while promoting competition for adhesion and immunomodulation.

Klebsiella pneumoniae (K. pneumoniae) is a multi-resistant opportunist pathogen able to colonize the human gut, with a high ability to form biofilm. In a study conducted by Lagraveille et al. (2018), the anti-biofilm activity of 140 species of Lactobacillus (supernatant cultures) was evaluated against K. pneumoniae. Of this total, the super-nant of 13 strains significantly impaired biofilm formation, including that of Lactobacillus plantarum (L. plantarum) CIRM653 – which was also able to disrupt K. pneumoniae preformed biofilms. The association of K. pneumoniae with L. plantarum CIRM653 showed reduced three-dimen-sional structures associated with a decrease in K. pneumoniae biomass. Research has shown that L. plantarum CIRM653 supernatant induced transcriptional modifications of K. pneumoniae biofilm-related genes, including down-regulation of quorum detection-related lsr operons and
overexpression of the type 3 pilus structure genes. Another in vivo study by Vieira et al. (2016) demonstrated that Bifidobacterium longum 5 can reduce K. pneumoniae infection in mice. This probiotic protected mice from K. pneumoniae lung infection, specifically by inducing secretion of pro-inflammatory cytokines and neutrophil recruitment, and decreasing bacterial load in the lung, thereby reducing lethality rates by 50%.

Recent studies by Xu et al. (2020) suggested that patients affected by COVID 19 should use probiotics to avoid secondary infections. Some patients with COVID-19 had intestinal microbial dysbiosis. The nutritional and gastrointestinal functions must be assessed in all patients. Nutritional support and application of probiotics are suggested to regulate the balance of the intestinal microbiota and reduce the risk of secondary infection due to bacterial translocation.

Annual mortality rates from infectious diarrhea are about 2.2 million. Children are more vulnerable to severe gastroenteritis, and group A rotavirus is the main cause of the disease. Studies by Gonzalez-Ochoa et al. (2017) demonstrated that the association of probiotics, such as Bifidobacterium and Lactobacillus species, in combination with prebiotics, showed an improved anti-rotavirus response by reducing infectiosity and increasing rotavirus-specific anti-IgA levels. In addition, these probiotics have been linked to a shorter duration and severity of diarrhea due to rotavirus infection, not only preventing the infectious process, but also contributing to a lower incidence of re-infections.

Evidence indicates that the pathogenic potential of Candida spp. also depends on their ability to produce biofilms on abiotic and biotic surfaces (Ribeiro et al., 2019). NYanz et al. (2014) investigated the anti-Candida activity of the crude extract of 13 different Lactobacillus strains. The authors found MIC values ranging from 1.25 to 10 mg/mL. Wannun et al. (2016) studied the antimicrobial activity of supernatant cultures of L. paracasei and identified a protein with a molecular weight of approximately 25,000 Da, which showed antimicrobial activity against Gram-positive, Gram-negative bacteria and yeast in in vitro microdilution tests.

Orsi et al. (2014) verified that the crude filtrate supernatants from L. acidophilus ATCC 314, L. rhamnosus ATCC 7469, L. plantarum ATCC 8014 and L. reuteri ATCC 55730 were able to affect both Candida albicans (C. albicans) hyphae formation and preformed and mature biofilm development. The inhibitory effects were Lactobacillus strain-dependent. L. plantarum, L. acidophilus and L. reuteri impaired biofilm formation and only L. plantarum and L. reuteri disrupted biofilm cells. Recent studies have also confirmed that Lactobacillus spp. can reduce C. albicans hyphae formation (Ribeiro et al., 2017; Santos et al., 2019) by releasing antimicrobial compounds.

Chew et al. (2015) used L. rhamnosus GR-1 and L. reuteri RC-14 to treat Candida glabrata (C. glabrata). Biofilm formation was evaluated by scanning electron microscopy (SEM) and genes related to biofilm formation were also analyzed. The SEM analysis revealed disrupted mixed biofilm cultures of C. glabrata and probiotic lactobacilli. In addition, the biofilm-related C. glabrata genes EPA6 and YAK1 were downregulated in response to the probiotic lactobacilli challenges.

Rossoni et al. (2017) evaluated the protective action of probiotics against C. albicans infection. Exposure to a dose of L. paracasei 28.4 activated the immune system of Galleria mellonella larvae, which may allow the larvae to modulate C. albicans infection. These results show that probiotics can affect the immune response of larvae. Other study performed by Rossoni et al. (2018) evaluated the inhibitory effects of probiotic microorganisms on three C. albicans strains. Thirty lactobacilli strains were isolated and tested for their antimicrobial activity against C. albicans biofilms in vitro. L. paracasei 28.4, L. rhamnosus 5.2 and L. fermentum 20.4 isolates exhibited the most significant inhibitory activity against C. albicans, disrupting biofilm development and retarding hyphal formation. qPCR analysis showed that the ALS3, HWP1, EFG1 and CPH1 genes were downregulated after treatment with the probiotic microorganisms. L. paracasei 28.4, L. rhamnosus 5.2 and L. fermentum 20.4 demonstrated antifungal activity through inhibition of C. albicans biofilms.

Liao et al. (2019) analyzed the effects of L. casei administration for vaginal candidiasis in an experimental model of C. albicans-infected mice. For the prophylactic test, the animals were submitted to vaginal inoculation of L. casei for 7 days. Next, the animals were infected with C. albicans into the vaginal cavity, and two days after the infection, all mice were euthanized, and the number of CFU/mL was determined. In the therapeutic assays, the animals were infected with C. albicans, and after 2 days, they received L. casei for five days. Next, the number of CFU/mL in the vaginal samples were determined. The results showed that prophylactic administration of L. casei was able to improve the immunity of vaginal mucosa, increasing the production of IL-17 during the infection. IL-23 levels were lower than those in the control group, showing that L. casei also had anti-inflammatory properties. Regarding to the therapeutic group, L. casei reduced the fungal vaginal burden after a 5-day treatment.

Krzysciak et al. (2017) studied L. salivarius as a possible probiotic candidate against mixed biofilm cultures of C. albicans and Streptococcus mutans (S. mutans), since this microbial association has been implicated in the progression of early childhood caries. The probiotic treatment reduced the biofilm mass and the number of S. mutans and C. albicans cells. Moreover, C. albicans cells treated with L. salivarius had their ability to form hyphae or germ tubes significantly impacted.

Poor skin conditions can affect the patient’s quality of life because of discomfort. Human skin is composed of numerous fungi and bacteria that live in symbiosis (Mottin & Suyenaga, 2018). Acne and Atopic Dermatitis (AD) are chronic skin conditions which require long periods of treatment and maintenance. Studies have shown that the use of probiotics in these cases has shown good results without adverse effects. In vitro studies have shown the capacity of probiotics, such as Streptococcus salivarius and Enterococcus faecalis, to directly inhibit P. acnes growth through the production of antibacterial proteins (bacteriocins) and immunomodulatory effects. Probiotics have been shown to have direct benefits (by inhibiting P. acnes) and indirect benefits (by decreasing the inflammatory response) (Robe et al., 2015; Bowe et al., 2006).

In a clinical study using Lactobacillus plantarum (L. plantarum) extract, Wu et al. (2012) observed a reduction in mild acne lesions, with amelioration of erythema and skin barrier reconstruction.

A study performed by Oh et al. (2006) reported an inhibitory effect of a bacteriocin produced by Lactococcus sp. HY 499 against S. epidermidis, S. aureus and P. acnes, in a patch test. The authors suggested the use of this bacteriocin produced by Lactococcus sp. HY 449 as an antimicrobial agent in cosmetic formulations. A lack of normal microbial skin diversity combined with an abundance of staphylococcal species in patients with atopic dermatitis further leads to disruption of skin-barrier homeostasis (Knaackstedt, Knaackstedt, & Gatherwright, 2020). The absence of allergic reactions and irritation consists of a great advantage for the use of probiotics compared to current treatments (Powers et al., 2015). The inhibitory effects on P acnes, S. epidermidis and S. aureus are relevant, as the same probiotic can be potentially used to treat different skin conditions.

Studies indicate that individuals with atopic dermatitis have an abundance of Staphylococcus aureus, when compared with individuals without the disease. Such an imbalance may be related to the breakdown of the skin barrier (D’Auria et al., 2016), which results in an ineffective protection from allergens and microorganisms.

A group of researchers led by Kawahara (Kawahara, Hanawa, & Sugiyama, 2018) evaluated the topical application of homogenized Lactobacillus reuteri in water and showed that there was significant suppression in the development of atopic skin lesions induced by mites and other pathogens.

In another study, Rosignoli et al. (2018) tested whether the topical application of Lactobacillus johnsonii could inhibit S. aureus adhesion to the skin and increase innate skin immunity. The authors demonstrated that application of this suspension reduced S. aureus adhesion by up to
74% and modulated endogenous expression of antimicrobial peptides (AMPs).

A study conducted by Navarro Lopes et al. (2018) determined the effectiveness of a mixture of oral probiotics. The authors showed a significant decrease in the SCORAD index [Scoring of Atopic Dermatitis (SCORAD) index] in the experimental group when compared to the control. In addition, they demonstrated that the probiotic mixture reduced the use of topical steroids in individuals with moderate atopic dermatitis (AD). This study corroborates the data found by Huang et al. (2017), who demonstrated that a mixture of Lactobacillus fermentus and Lactobacillus salivaris reduced the SCORAD scores in individuals aged 1 to 18 years.

As for atopic dermatitis and seborrheic dermatitis, topical probiotics have demonstrated the ability to increase skin ceramides, improve erythema, flaking and itching and reduced S. aureus microbial load. However, studies have used different probiotics, vehicles and dosage and investigated several parameters. The most used probiotics were S. thermophiles, V. filiformis, S. hominis, S. epidermidies and L. johnsonii (Di Marzio et al., 2003; Blanchet-Réthoré et al., 2017; Knackstedt et al., 2020).

Thus far, several studies have evaluated the action of probiotics against fungal and bacterial. Although the results are quite surprising, the exact molecular mechanisms by which probiotics can inhibit pathogenic strains remain largely unknown and speculative. Thus, for future studies, new approaches should be developed to improve probiotic research. Even though probiotics are considered safe, additional studies are needed to improve the exact composition and routes of administration.

5. Probiotic encapsulation for delivery to the action site

The benefits of probiotics to human health are unequivocal. However, for these beneficial bacteria to exercise their biological activities effectively, the number of viable organisms must be greater than or equal to $10^7$ CFU/mL or gram of product in use (Rossier-Miranda et al., 2010; Serna-Cock & Vallejo-Castillo, 2013). In addition, the survival of probiotic microorganisms can be affected by different factors (e.g., pH, temperature, peroxide production etc.), which makes delivering viable cells to the place where they should exercise their action a challenging task. Thus, the study and investment in technologies for probiotic encapsulation is much needed (Gbassi & Vandamme, 2012).

Drug encapsulation technologies have been studied over time and shown significant benefits, such as increased therapeutic efficacy and reduced dose-dependent toxicity (Singh, Hemant, Ram, & Shivakumar, 2010). To date, nanotechnology provides different viable nanocarrier options for preserving and delivering drugs, such as liposomes, micelles, carbon nanotubes and dendrimers (Kumari, Singla, Guliani, & Yadav, 2014).

In addition to drugs, living cells (e.g., probiotic microorganisms) can also be encapsulated. Common nanocarriers used for drug delivery may not serve for cell encapsulation. Instead, a biocompatible matrix should be employed to encapsulate and immobilize viable cells protecting them from a hostile environment, such as chemical and physical stress and the host’s immune response (Orive, Santos, Pedraz, & Hernández, 2013). The biocompatible matrix should act as a semipermeable membrane, allowing bi-directional transport of nutrients (Griffith & Naughton, 2002, Gurruchaga et al., 2015). Thus, the cells can remain viable and produce therapeutic substances that will be delivered more than once to the site of action by permeating through the polymer matrix (Fig. 3).
The effectiveness of cell encapsulation depends on the matrix polymers, which can be obtained from natural sources (polysaccharides, polypeptides and polynucleotides) or manufactured. Different biocompatible materials have been used to immobilize cells in a matrix, such as hyaluronic acid, fibrin, agarose and collagen (Vrana et al., 2009). Table 3 shows a selection of recent studies that carried out the encapsulation of probiotic microorganisms to assess their antimicrobial potential. Alginate was considered the most studied and appropriate biomaterial due to its biocompatibility and ease of handling (Vrana et al., 2009). It has been shown that alginate can pass through stomach acids without any degradation, with the spheres formed by this gel reaching the intestine satisfactorily (Rayment et al., 2009). Probiotic encapsulation with alginate was compared to the formation of a “beneficial” biofilm by these bacteria (Li et al., 2018).

Although probiotics have been extensively studied in recent decades, their biological activity remains little explored (Fig. 4). In addition, the number of probiotic encapsulation studies is well below the total number of studies on encapsulation systems. Fig. 4 shows the number of published articles reporting encapsulation systems (red line) as compared to antimicrobial activity of probiotics (green line) and other biological activities of probiotics (blue line). Encapsulation of probiotic microorganisms to treat infectious conditions other than intestinal infections may open new avenues for alternative antimicrobial therapies as well as adjuvant and/or synergistic approaches with conventional antibiotic therapy.

### 6. Probiotics-conventional drugs synergism for antimicrobial therapy

While the antimicrobial drugs currently used in clinical practice are effective, they have a high cost, side effects and therapeutic resistance (Graham & Fischbach, 2010; Vitor & Vale, 2011). For this reason, the combination between probiotic microorganisms and conventional drugs has been considered. The advantages of this synergism include: (i) faster healing; (ii) half dose of conventional drug needed; (iii) reduction of side effects caused by classical therapy; and (iv) increasing eradication rates of some microbial infections (Wolvers et al., 2010; Kosgey et al., 2019).

Lesbros-Pantoflickova, Corthesy-Theulaz, and Blum (2007) reviewed nine studies addressing the benefits of co-administration of probiotics with antibiotics for the treatment of H. pylori infections, particularly in the prevention of side effects and improvement of eradication rates. Probiotics administered together with standard therapy (two antibiotics and a proton pump inhibitor) for H. pylori infection achieved an eradication rate of 81%, as compared to 71% of conventional therapy alone. Consistent with this, the probiotics-conventional drugs synergism promoted reduction in H. pylori therapy-associated side effects (23% vs 47%, synergism vs conventional therapy alone) (Lesbros-Pantoflickova et al., 2007).

As shown in Table 4, some studies have validated the beneficial effect of synergism between probiotics and conventional therapies for the treatment of fungal and bacterial infections. A report by Russo et al. (2018) evaluated the effectiveness of an oral formulation containing probiotics with lactoferrin glycoprotein as an adjuvant therapy to topical clotrimazole for vulvovaginal candidiasis (VVC) episodes. Key findings showed that the investigated probiotics (Lactobacillus acidophilus GLA-14 and Lactobacillus rhamnosus HN001) and the lactoferrin mixture administered simultaneously with the antifungal drug (clotrimazole) was able to reduce the symptoms and recurrence of VVC. The authors highlighted the administration of probiotics and lactoferrin after conventional therapy with topical clotrimazole as a potential maintenance treatment that reduced candidiasis relapse at a 3- and 6-month follow-up. Similarly, Martínez et al. (2009) evaluated whether lactobacilli improved the efficacy of fluconazole in patients with VVC. The results evidenced the importance of adjuvant treatment with probiotics (L. rhamnosus GR-1 and Lactobacillus reuteri RC-14) that were added to a single dose of 150 mg fluconazole during a 4-week therapy for VVC. The effectiveness of the combination between probiotics and an antifungal drug was validated based on the rate of culture-free women (38.5%) as compared to that of women who received only fluconazole (10.3%). Evidence shows that probiotics represent an alternative and effective approach for the treatment of VVC, in view of...
their ability to resist Candida spp. and maintain or recover the normal vaginal microbiota (Matsubara et al., 2016). Additionally, the co-aggregation between lactobacilli and Candida spp. prevents the binding of yeast cells to receptors on the vaginal epithelium, thereby blocking an important virulence factor of Candida species – host-yeast adhesion (Reid et al., 2003; Reid & Hammond, 2005).

Some studies have investigated the synergistic effect of probiotics with conventional antifungal drugs for the treatment of Candida spp. infections. However, the potential of probiotics as an adjuvant to conventional antifungal therapy for other highly prevalent invasive fungal infections remains to be determined in future research.

The administration of beneficial microorganisms in the form of probiotics as an adjuvant treatment has also been a valuable approach for the therapy of chronic periodontitis (CP). Ikram et al. (2019) reported an evaluation and comparison of the clinical efficacy of administration of probiotics containing L. reuteri as an adjuvant to scaling and root planing (SRP) for the treatment of CP. Research findings demonstrated that the adjunctive use of L. reuteri was effective in resolving inflammation and improving periodontal outcomes through improvement of the clinical periodontal parameters, including plaque index, bleeding on probing, periodontal pocket depth, and clinical attachment level gain.

Collectively, it is possible to affirm that the use of probiotics are presently being investigated as an adjuvant innovative treatment to current antimicrobial agents, but these novel therapeutic strategies remain to be further tested and validated.

7. Final considerations

The human body is the target of several pathogenic microorganisms, such as S. aureus, MRSA, P. aeruginosa and C. albicans. This review provided unequivocal evidence on the antimicrobial activity of probiotics against clinically relevant pathogens. Nonetheless, while some molecules have been considered responsible for the antimicrobial activity of probiotics (e.g., biosurfactants, hydrogen peroxide, lactic acid, acetic acid and bacteriocins), further studies should characterize new molecules and elucidate their inhibitory mechanisms against pathogenic fungal and bacterial strains. Probiotic research needs to be bio-guided during the whole process until they enter the clinical phase. Currently, there is no consensus or standardization for the clinical use of probiotics as an antimicrobial therapy, and the definition of dosage, mechanism of action and clinical efficacy remain to be determine. Lastly, the use of nanotechnologies to encapsulate molecules from probiotic extracts should be encouraged for the development of more effective therapeutic approaches.

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Ethical statement

The authors declare that this is a literature review work. Thus, it does not imply human or animal experimentation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.


