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Review

Probiotics as Therapeutic Tools against Pathogenic Biofilms: Have We Found the Perfect Weapon?

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Abstract: Bacterial populations inhabiting a variety of natural and human-associated niches have the ability to grow in the form of biofilms. A large part of pathological chronic conditions, and essentially all the bacterial infections associated with implanted medical devices or prosthetics, are caused by microorganisms embedded in a matrix made of polysaccharides, proteins, and nucleic acids. Biofilm infections are generally characterized by a slow onset, mild symptoms, tendency to chronicity, and refractory response to antibiotic therapy. Even though the molecular mechanisms responsible for resistance to antimicrobial agents and host defenses have been deeply clarified, effective means to fight biofilms are still required. Lactic acid bacteria (LAB), used as probiotics, are emerging as powerful weapons to prevent adhesion, biofilm formation, and control overgrowth of pathogens. Hence, using probiotics or their metabolites to quench and interrupt bacterial communication and aggregation, and to interfere with biofilm formation and stability, might represent a new frontier in clinical microbiology and a valid alternative to antibiotic therapies. This review summarizes the current knowledge on the experimental and therapeutic applications of LAB to interfere with biofilm formation or disrupt the stability of pathogenic biofilms.

Keywords: lactic acid bacteria; biofilms; probiotics; quorum sensing; antibiotic resistance

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1. Introduction

Pathogenic bacterial biofilms are becoming one of the main concerns of the antibiotic era [1,2]. Biofilms are assemblages of microorganisms and the extracellular products they produce, that adhere on biotic or abiotic surfaces and are characterized by highly specialized interactions between them [3]. Biofilm-forming bacteria are embedded in a matrix of self-produced slime, constituted by extracellular polymeric substances (EPS) [4]. This growing mode can alter bacterial biological and physiological characteristics, such as reproduction, growth, gene transcription rate, and resistance towards antibiotics [5–7]. Schematically, the formation of a differentiated biofilm requires five maturation stages: (i) initial attachment of planktonic bacteria (reversible) to a surface; (ii) production and secretion of EPS and/or other means of docking, and specific adhesins (e.g., flagella, autotransporter proteins, fimbriae, curli fibers, and F-type conjugative pilus) that drive the transitional attachment from reversible to irreversible [8–10]; (iii) early-maturing of biofilm architecture as a super cellular structure; (iv) late-maturing of micro-colonies and evolution into a mature biofilm; and (v) detachment of cells from the biofilm and dispersion

into the surrounding environment (Figure 1). All these processes are strictly regulated by different cell-to-cell signaling molecules responsible for population density-dependent gene expression that can deeply affect the process of biofilm formation [11,12].

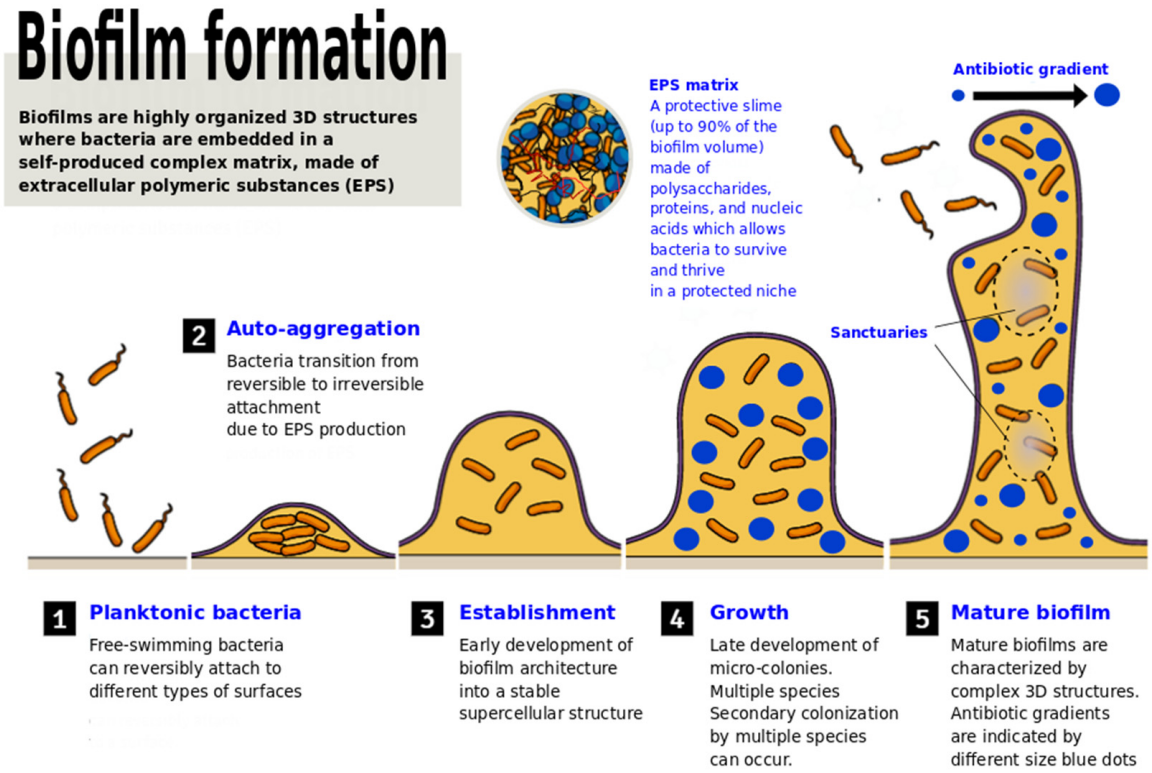

Figure 1. Schematic representation of the different steps required for the formation of a mature biofilm. The small and large blue dots represent areas with different antibiotic concentrations (denoting the presence of a gradient), and the grey zones are "sanctuaries" where bacteria can survive with a low concentration of antibiotics, which can favor the development of resistance.

The production of the EPS matrix, composed of polysaccharides, proteins, and nucleic acids (extracellular DNA—eDNA) allows for bacterial survival and proliferation in a protected niche with a constant nutrient supply and protection from the host immune system, disinfectants, and antibiotics [13,14]. Biofilms act as physical barriers, allowing bacteria to elude both immune detection and phagocytosis, while expressing genetic switches (or response regulators) that disturb immune cell activity [15]. Up to 80% of chronic infections worldwide are linked to biofilms and/or are caused by antibiotic resistant bacteria. Indeed, bacteria growing in a biofilm can be 100–1000 times more drug resistant compared to their planktonic counterpart [16].

The Antibiotic Resistance Threats Report (2019 AR Threats Report) by the American Centers for Disease Control and Prevention (CDC) reports that "more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result" [17]. The spread of antibiotic-resistant bacterial clones is a global threat to public health. The reasons behind this phenomenon span from unregulated antibiotic usage in livestock farming to malpractices or improper use of antibiotics in the treatment of human infections [18,19]. Different studies have shown that physicians tend to overprescribe antibiotics mainly due to pressure from patients or from the healthcare system, as well as financial incentives and attempts to maximize the number of patients treated. On the other hand, patients' lack of knowledge and awareness, access to antibiotics without a prescription, or premature stopping of antibiotic therapies as a consequence of improved health conditions, are other resistance promoting factors [20–24].

Several in vivo and in vitro studies have shown that LAB possess the ability of contrasting biofilm formation and growth. LAB are probiotics and are not prone to trigger

or promote the evolution of resistant pathogens. According to the European Food Safety Authority (EFSA), an important requirement of probiotics is, indeed, that they must not have antibiotic resistance genes which could spread through plasmids or transposons. Among LAB, members of the genera *Lactobacillus* and *Bifidobacterium* have emerged as the most commonly used probiotics [25].

In the majority of the scientific works on topical and oral probiotics, it is common to encounter a precise definition, originally given by World Health Organization (WHO): "Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host" [26,27]. To this clear statement corresponds a wide range of well-recognized and largely unquestioned "benefits" (e.g., recolonization of surfaces depleted of commensal bacteria after an antibiotic treatment, capacity to contrast and outcompete the growth of pathogenic microorganisms), plus a wider spectrum of unspecified, off-target, long-lasting, and sometimes, highly debated extra advantages (e.g., anti-carcinogenic effects, immune system modulation, mitigation of side effects of medicaments or invasive therapies). As a matter of fact, probiotics are often administered orally, but the benefits are not restricted to the gastrointestinal tract; changes and interactions affecting the microbiota of the skin, urinary tract, and mouth are well documented and indicative of broad range effects [28–30].

A relevant issue linked to the specific definition of probiotics reported above regards the quantification of the "sufficient amounts". Despite the difficulty in defining this parameter, probiotics are commonly regarded as safe and are administered as billions of microbial cells. Although monitoring and continuous surveillance, as well as precaution, are mandatory, probiotics have the advantage of presenting no (or limited) side effects linked to overdosage [31,32]. Recently, Barzegari et al. (2020) have evidenced the possibility of using probiotics and their derivatives against biofilms and encouraged in vivo studies to define the best strain-related antibiofilm activity [33].

Although research on the topic is very active, further studies are needed to gain insights into the mechanisms by which probiotics and their metabolites can be used and properly applied to manage biofilm infections in humans.

This review is centered on *Lactobacillus* and *Bifidobacterium* genera, and the molecules they produce (surfactant, bacteriocins and other metabolites), able to prevent and contrast the formation, or even dissolve, biofilms of pathogenic microorganisms. Therefore, we focused on the possibility of using these probiotics as prophylaxis or therapeutic agents against pathogenic biofilms.

2. Biofilms: The Good, the Bad, and the Ugly

Among the broadly accepted beneficial effects of topical and oral probiotics, there is the capacity to prevent or contrast the adherence, colonization, and reproduction of pathogens [34]. As previously mentioned, biofilm-forming pathogens colonizing human tissues, prosthetics, or other medical devices are normally more resistant to antibiotics and disinfectants. Lately, they are arising concern, especially in nosocomial settings, for their increasing resistance to last generation antibiotics as well [35,36]. Another common phenomenon that deserves a high level of surveillance is the development of mixed-species biofilms [37]. In such a complex context, microorganisms compete and cooperate in an unpredictable way; in some cases, multispecies biofilm infections can lead to worse outcomes compared to mono-species infections [38,39]. Indeed, the EPS matrix can confer physical protection from the penetrance of pharmaceutical compounds aimed to contrast bacterial reproduction and survival. Meanwhile, transcriptomic and metabolomic studies have identified hundreds of genes and metabolites that are differentially expressed by bacteria in the biofilm growing mode. These molecules have been associated with key mechanisms and pathways governing biofilm formation and maintenance, such as quorum sensing, ABC transporters, the two-component system, and amino acid metabolism [40,41]. Biofilms constitute a rather heterogeneous environment, where the community of microorganisms is distributed over a wide volume of space with different thickness. This implies

that an eventual exposure to antibiotics does not occur in a homogenous manner, but rather through a gradient, and that in the inner part of the matrix there could be some "sanctuaries", or shielded areas, that provide protection and time for developing adaptive resistance to a low concentration of antibiotics [42]. Antibiotic gradients are known to promote the development of resistance, so that the bacteria can rapidly evolve the capacity to survive in areas with higher antibiotic concentration [43].

In general, biofilms have been historically associated with pathogenic bacteria and seen as a negative phenomenon. However, probiotics, particularly LAB such as *Lactobacillus* spp. and *Bifidobacterium* spp., grow either in planktonic form or as biofilms. LAB are Gram-positive rods and cocci that present low G + C content and are non-sporulating. They share many biochemical, physiological, and genetic properties and are part of the autochthonous microbiota of several body niches (e.g., gastrointestinal tract, vagina), and found in many types of fermented food [44]. Traditional fermented foods are rich sources of LAB with probiotic characteristics [45–47]. The host mucosal surfaces, in particular the gut, can be stably or transiently colonized by such probiotics. The capacity of these microorganisms to colonize substrates and form biofilms is still waiting to express its full potential and gain broader application for human health and food safety. Indeed, new species delivered into an environment that fail to form biofilms can be eliminated quickly, even when delivered in abundance; this aspect might be a major cause behind the low efficiency of some probiotic combinations [48].

Lactobacillus species that form biofilms are commonly reported in different kinds of probiotics, such as *L. rhamnosus*, *L. plantarum*, *L. reuteri*, and *L. fermentum*. A growing body of evidence supports the advantages of probiotic strains in biofilm form (e.g., increased resistance to temperature, antibiotics, gastric pH, and mechanical stress) compared to bacteria in the planktonic lifestyle [49]. Biofilms formed by different strains of *Lactobacillus plantarum* (now *Lactiplantibacillus plantarum*) and *Lactobacillus fermentum* (now *Limosilactobacillus fermentum*) have been accurately evaluated in vitro and were found to be associated with the production of anti-inflammatory molecules inhibiting the growth of pathogens; in vivo efficacy was demonstrated as well [50]. Remarkably, the reported beneficial effects were highly variable and strain dependent, and more importantly, such events were not registered in the planktonic form [51].

3. Methods: Dataset and Databases Used for Literature Searching

A literature analysis was accomplished considering reviews and scientific articles published in the PubMed, ScienceDirect, Web of Science, and Scopus databases. We included only contributions published in English, giving more attention to recent articles written in recent decades (2000–2021), but also including older works, especially when describing well-established laboratory practices. The search query was carried out by including the following keywords: "probiotics", "prebiotics", "LAB", "lactic acid bacteria", "novel antibiotics", "biofilm", "quorum sensing", "quorum quenching", and "antibiotic resistance". We organized and summarized the results in three tables, which contain important information on the different methods used to study biofilms, the main mechanisms and quorum sensing molecules used by bacteria to communicate within biofilms, and the mechanisms used by LAB to contrast pathogenic biofilms.

4. Methods for the Detection and Evaluation of Antibiofilm Activity

There are several methods used to screen and quantify biofilm formation and antibiofilm activity: Congo red agar (CRA), plate counting of biofilm-embedded bacteria (sessile bacteria), qPCR, mass spectrometry (MS), confocal laser scanning microscopy (CLSM), and others [52]. The most common and widely used method to study biofilms is a microtiter plate test, which involves staining biofilm forming bacteria on microplate surfaces by either crystal violet or safranin, respectively, for Gram-positive or Gram-negative bacteria. CRA is generally used to determine slime production; therefore, it can be considered an indirect approach to evaluate biofilm formation. Antibiotic susceptibility and the

biofilm-forming activity of bacteria are commonly and easily assessed by disc diffusion and crystal violet assays, respectively [53]. More recently, transcriptomics and metabolomics are giving important insights for a proper characterization of biofilm-embedded bacteria [40].

In vitro models are thought to address fundamental questions about biofilm formation, genetic regulation, spatial architecture, distribution of metabolic products and nutrients, cellular density, and production/release of EPS [54]. Currently these models are classified in three categories: (i) static models (or static), (ii) dynamic systems (or open), and (iii) and microcosms (Table 1). Static models are characterized by limited nutrient and gas gradients. This category includes some of the most useful models, such as microtiter plates and CRA, which allow rapid quantification of biofilm biomass—through crystal violet or safranin staining of viable cells—through a MTT reduction assay [55]. All dynamic models are characterized by continuous circulation of fresh culture medium that replaces spent medium, allowing for the elimination of waste metabolic products and of dispersed and dead cells. These models have the advantage of allowing for control of environmental parameters (e.g., physical and chemical factors), which maximizes the production of biofilm biomass but requires specialized equipment and technical skills [56]. Microcosm models are more complex and sophisticated since they are specifically designed to more accurately mimic in situ conditions. In general, they are based on the use of a human cell monolayer covered with bacteria directly isolated from human samples, therefore comprehending a microbiota that is more variable and difficult to characterize [57,58].

Table 1. This table briefly presents the three different methods to study biofilms, with some extra details of properties, field of application, and advantages.

Models	Properties	Uses	Advantages	References
Static systems				
Colony biofilm	Colonies grow over agar, maintenance of basic biofilm characteristics (e.g., chemical gradient)	Antibiotic susceptibility assay	Simple and reproducible, high throughput	[59,60]
Microtiter plate	Most widely used, bacterial adhere to well surfaces	Semiquantitative evaluation of biofilm formation of strains, biofilm antibiotic tolerance test, study of antibiofilm efficiency	Simple to perform, molecular genetic tests are allowed, high throughput	[61,62]
Biofilm ring test	Use of magnetic beads to immobilize bacteria	Quantitative evaluation of biofilm formation of strains	Rapid monitoring of biofilm formation, investigation of early adhesion	[63]
Calgary biofilm device	Use of a lid with 96 pegs on which biofilms develop	Biofilm antibacterial tolerance and resistance, efficiency of antibiofilm/antibiotic products	Pegs are individually removable, avoiding cross contamination	[64]
Open systems				
Flow cell	Flat walled transparent chambers continuously sprinkled with medium, automatic system	Evaluation of biofilm formation in real-time (chamber is under microscope), efficiency of antibiofilm/antibiotic products	Continuous image record, single cell observation	[65]
Microfermentors	Chemostat-based, biofilms are formed over a removable spatula (mode of different materials)	Evaluation of biofilm formation of strains, efficiency of antibiotic products	Lare scale biofilm biomass production; genetic, biochemical, and microscopic analyses are allowed; easy conversion into microcosms	[66]
Modified Robbins device	Linear rectangular array of ports in which plugs are inserted	Artificial throat used to evaluate the efficiency of product in rubber trachea-oesophageal prostheses	Each plug can be removed individually and aseptically	[67]
CDC biofilm reactors	Consists of eight polypropylene coupon holder rods suspended from a polyethylene ported lid	Evaluation of biofilm formation, antibiotic resistance and tolerance; study of biofilm over time	Easy sampling event at different time	[68]

Table 1. *Cont.*

Models	Properties	Uses	Advantages	References
Kadouri system	Based on microtiter plate assay with continuous medium replacement	Testing multiple nutritional condition and treatments	Formation of high amount of mature biofilm in wells	[69]
Rotating disc reactor	Rotor embedded with a magnetic stir holding 6 to 24 coupons over which biofilms will form	Used to study multispecies biofilm; evaluation of antibacterial molecules	Modification of liquid shear forces over the coupons	[70]
Microfluidic biochips	Chip located in aluminium support in which dielectric sensors control temperature	Quantitative cell and population analyses	Measurement of biofilm growth and maturation with high sensitivity	[71]
Drip flow reactors	Consists of four test channels, each holding one standard glass microscope slide sized coupon	Evaluation of antimicrobial and antibiofilm substances; study biofilm heterogeneity	Establishment of both solid-air and solid-liquid interfaces	[72]
Microcosms				
Reconstituted human epithelia (RHE)	Human keratinocytes (from buccal mucosa) serve as surface to growth biofilm	Oral biofilm formation	Possibility to study oral receptor specificity	[73]
Microfluidic co-culture model	HeLa cells covered with microfluidic channels over which biofilm forms	Mimic gastrointestinal environment	Real-time visualization of biofilm growth	[74]
Endothelial cells under flow model	Microvascular endothelial cells are attached on microscope slide allowing biofilm development	Monitoring of blood vessel microenvironment and biofilm formation dynamics	Biofilm formation stages and cell can be stained with fluorescent dyes and monitored	[75]
Airway epithelial cell model	Collagen coated membranes allow growth of airway epithelial cells for biofilm development	Oral biofilm formation (cystic fibrosis, chronic rhinosinusitis)	Investigation of air-liquid biofilm model	[76]

In general, the antimicrobial activities of probiotic combinations are evaluated by agar diffusion. To distinguish between isolates with bacteriostatic or bactericidal activity, further tests can be performed using the agar overlay method. This method consists of a double layer of agar with different densities to allow the diffusion of metabolites from probiotic to pathogenic bacteria plated over the probiotic in soft agar (0.5% w/v). The practice of calculating the MIC (Minimum Inhibitory Concentration) in studies evaluating the inhibitory activity on biofilms of probiotics or their metabolites is not so common. However, this parameter remains extremely important for comparative purposes.

As a matter of fact, surface-attached biofilms remain difficult to study. Dynamic models for investigating in vitro biofilm formation might present some advantages over static systems [77]. Such systems are derived or modified from static assays to favor better biofilm growth and to study the ability of biofilm-forming bacteria to adhere to surfaces. To give an overview of these methods, we could mention the use of a rotating platform which provides shear of an embedded cover slip that can be removed and examined by CLSM microscopy. Another dynamic method consists of using numerous glass beads in a flask incubated with shaking on a rotating platform. The surface area for biofilm formation is increased, and this approach is suitable for harvesting high amounts of cells for transcriptomic or proteomic investigations.

To study biofilm development and the different developmental stages in real-time, the elected methods are flow-cell systems. Using CLSM, the biofilm can be monitored non-invasively and continuously, since the bacteria are grown in small channels on a glass surface.

Metabolomics is providing information on the spatial and temporal evolution of the metabolic state during biofilm formation. As an example, it was possible to describe two different strains of *Helicobacter pylori* based on the production of metabolites, since

low-biofilm-formers produced more metabolites than high-biofilm-formers [78]. Furthermore, liquid chromatography coupled with mass spectrometry (LC-MS) revealed and disclosed the biological and metabolic processes, plus a specific proteomic profile, essential for *Candida albicans* biofilm growth [79]. Biological "omics" and computational approaches (including in silico techniques, such as virtual screening and machine learning), are emerging as powerful tools for the discovery of candidate agents with antibiofilm activity. Finally, organoids, in vitro 3D multicellular systems mimicking the corresponding in vivo organ, represent a realistic biofilm model to test the ability of newly discovered molecules to interfere with key biofilm regulators [80].

5. The Battle of LAB against Pathogenic Biofilms

5.1. How Lactobacillus May Contrast Biofilm Formation and Stability

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multi-drug resistant (MDR) microorganism and one of the principal nosocomial pathogens worldwide [81]. Different strains belonging to the genus *Lactobacillus* (as well as *Bifidobacterium*) isolated from various sources have been shown to contrast the growth of *S. aureus* and even of clinical isolates of MRSA in vitro [82]. Their effects were mediated both by direct cell competitive exclusion and the production of short chain fatty acids or bacteriocin-like inhibitors. In addition, *L. acidophilus* was also reported to inhibit *S. aureus* biofilm formation and lipase production. In another study, *L. fermentum* TCUESC01, isolated from cocoa seeds, was shown to effectively inhibit *S. aureus* biofilm formation. The inhibition mechanism was based on the release of soluble molecules which suppressed the expression of two genes (*icaA* and *icaR*) with an important role in biofilm synthesis [83].

MDR *Proteus mirabilis* isolates show low antibiotic susceptibility and biofilm-forming activity that can cause serious urinary tract infections [84]. A recent study demonstrated that cultures and cell-free supernatants of *L. casei* DSM 20011 and *L. reuteri* DSM 20016 exhibited strong antimicrobial, anti-adherence, and antibiofilm formation activities against MDR *P. mirabilis*. In addition, supernatants of *L. casei* and *L. reuteri* significantly reduced mature biofilm formation and adherence (>60% compared to controls), indicating that these species of lactobacilli could be utilized to combat *Proteus*-associated urinary tract infections [85].

Dental caries has multifactorial causes and arises from an imbalance between the host and the microbiota of the mouth. For a long time, *Streptococcus mutans* in its biofilm form has been known to contribute to dental caries formation significantly; recently, the one pathogen –one disease approach has been deeply challenged, and the concurrent role of the entire microbiota in the health of the oral cavity tends to be more prominent [86]. The capacity of different *Lactobacillus* species to inhibit growth, biofilm formation, and gene expression of *S. mutans* has been evaluated. Susceptibility testing indicated antibacterial (pH-dependent) and antibiofilm activities of *L. casei* (ATCC 393), *L. reuteri* (ATCC 23272), *L. plantarum* (ATCC 14917), and *L. salivarius* (ATCC 11741) against *S. mutans.* All *Lactobacillus* species previously mentioned contrasted and limited the growth and virulence of *S. mutans.* Reduction in microcolony formation and exopolysaccharide structural changes were also highlighted by scanning electron microscopy. The highest antimicrobial activities were reported for *L. casei* and *L. reuteri*, whereas the lowest antimicrobial activities were observed with *L. plantarum* and *L. salivarius*. The highest antibiofilm and peroxide-dependent antimicrobial activities were reported for *L. salivarius*. Reduced expression of genes involved in exopolysaccharide production, acid tolerance, and quorum sensing were reported for all biofilm-forming cells treated with *Lactobacillus* spp. supernatants [87]. In a study on mixed biofilm formation by fungi and bacteria on silicone in vitro, *Lactobacillus* supernatant showed high efficiency against both microorganisms [88]. In the field of oral infections, the probiotic strain *L. brevis* CD2 was shown to inhibit the opportunistic anaerobe *Prevotella melaninogenica* (PM1), a well-known causative agent of periodontitis. The inhibitory effect of *L. brevis* CD2 on *P. melaninogenica* PM1 biofilms was evaluated in vitro using two different methods: the anaerobe was exposed to the supernatant of the strain

in one case, or the two microorganisms were grown together to obtain single or mixed biofilms, in the second case. The inhibitory effect of CD2 on PM1 was also checked by the agar overlay method. The development of PM1 biofilm was strongly affected (56% decrease in OD_{570} value) by the CD2 supernatant after 96 h—with a dose-dependent biofilm reduction using several supernatant dilutions. Confocal microscopy on the mixed biofilms revealed the ability of CD2 to prevail over PM1, greatly reducing the biofilm of the latter. The authors hypothesized that the strong adherence ability of the CD2 strain and the release of metabolites may be responsible for reducing the PM1 biofilm [89].

The use of antibiotics for the treatment of cholera is associated with side effects, such as gut dysbiosis, due to the depletion of beneficial microbiota and the risk of spreading antibiotic resistance; hence, the search for alternative therapeutic agents is extremely active. Different strains of *Lactobacillus* spp., screened and isolated from fecal samples of healthy children in cholera endemic area, were tested for their abilities to prevent biofilm formation and to disperse the preformed biofilms of *Vibrio cholerae* and *V. parahaemolyticus*. The results showed that the culture supernatant (CS) of seven isolates of *Lactobacillus* spp. used in the study inhibited the biofilm formation of *V. cholerae* by more than 90% [90].

A recent study showed the role of *L. gasseri* in contrasting the adhesion of the protozoan parasite *Trichomonas vaginalis* to host cells, a critical virulence aspect of this pathogen [91]. The aggregation-promoting factor-2 (APF-2) produced by *L. gasseri* ATCC 9857 was found to be highly inhibitory in the adhesion of *T. vaginalis* to human vaginal ectocervical cells. This important finding highlights that lactobacilli remain of key importance for the development of specific therapeutic strategies, even towards non-bacterial pathogens.

As a matter of fact, probiotics are active against non-bacterial biofilms as well. For example, *C. albicans* biofilm is associated with denture-related stomatitis and oral candidiasis, especially in elderly people. A study investigating a *C. albicans* biofilm on a denture base resin treated with *L. rhamnosus* and *L. casei* showed that the probiotics' surfactant exhibited strong antifungal activity against blastoconidia and biofilm of *C. albicans*. Even when the *C. albicans* biofilm was already formed and sequentially treated with *L. rhamnosus* and *L. casei*, inhibition of the biofilm on the denture surface was reported [92]. Therefore, *L. rhamnosus* and *L. casei* probiotics could have practical applications for preventing and treating denture-related stomatitis and other *Candida* infections, even in neonates [93,94].

It is not uncommon to register discrepancies between the effectiveness of probiotics in vitro and in vivo. Therefore, in vitro antimicrobial activity does not necessarily assure efficacy in animal infectious models. However, cases in which the in vitro and in vivo results were congruent are also reported. As an example, *L. plantarum*, which showed the highest inhibition activity against *S. aureus* in vitro, was also very effective topically in preventing skin wound infection in *S. aureus*-infected mice. Bacteriocin-producing *Lactobacillus sakei* 2a has been shown to protect gnotobiotic mice against experimental challenge with *L. monocytogenes* [95]. A recent study aimed at evaluating the effects of *Lactobacillus* administered intranasally on a murine model of *P. aeruginosa* pneumonia (strain PAO1). Two probiotic combinations were selected for in vivo testing (1-L.rff for *L. rhamnosus* and two *L. fermentum* strains, and 2-L.psb for *L. paracasei*, *L. salivarius*, and *L. brevis*) out of 50 clinical isolates screened for the ability to decrease the synthesis of two PAO1 produced QS-dependent virulence factors (elastase and pyocyanin). Intranasal priming with both probiotic blends acted as a prophylaxis and avoided fatal complications caused by PAO1 pneumonia in mice, showing encouraging results to move towards clinical trials [96].

5.2. How Bifodobacteria May Contrast Pathogenic Biofilms

Among the Bifidobacteria, *Bifidobacterium bifidum* BGN4 is a widely used probiotic strain that has been included as a major ingredient to produce nutraceutical products for the last 20 years [97]. The various bio-functional effects and potential for industrial application of *B. bifidum* BGN4 have been characterized and proven in vitro (i.e., phytochemical bio-

catalysis, cell adhesion, anti-carcinogenic effects on cell lines, and immunomodulatory effects on immune cells) and in vivo experiments (see below).

A study investigated the effect of *Bifidobacterium* spp. on the interference with the production of quorum-sensing (QS) signals and biofilm formation by enterohemorrhagic *E. coli* (EHEC) O157:H7. In an AI-2 bioassay, cell extracts of different *Bifidobacterium* reference strains (*B. longum* ATCC 15707, *B. adolescentis* ATCC 15706, and *B. breve* ATCC 15700) were rather effective; they resulted in a 36% reduction in biofilm formation. Cell extracts of *B. longum* ATCC 15707 were also able to reduce the virulence of EHEC O157:H7 in the *Caenorhabditis elegans* nematode in vivo model [98]. Another study highlighted how *B. lactis* and *B. infantis*, alone or in combination, have an antagonist effect on biofilms of periodontopathogens, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, but minimal influence on *Streptococcus oralis* growth in vitro [99].

Bifidobacteria strains are often used in probiotic combination with other LAB. One of these combinations, constituted of *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* 99, and *P. freudenreichii* JS was shown to inhibit pathogen adhesion (including *Salmonella enterica*, *Clostridium difficile*, *L. monocytogenes*, and *S. aureus*) to human intestinal mucus (in vitro). The same combination with another bifidobacterial strain (*B. lactis* Bb12) was less effective [100].

The studies regarding the ability of Bifidobacteria to contrast pathogenic biofilms are not so numerous as the ones on lactobacilli. Some experimental works have also highlighted a lower effectiveness compared to other LAB. As an example, Miyazaki et al. (2010) highlighted that CS of a *Lactobacillus* strain has a strong bactericidal effect on auto aggregative *E. coli*, while no effect was reported for *Bifidobacteria* [101]. Discrepancies among laboratory results and experiments in animal models are known for Bifidobacteria as well. For example, the *S. aureus* 8325-4 strain was shown to be sensitive in vitro to *L. acidophilus*, while *B. bifidum* best inhibited experimental intravaginal staphylococcosis in mice caused by the same bacteria [82]. For *B. bifidum* BGN4, a wide spectrum of beneficial effects in vivo (i.e., suppressed allergic responses in mouse model and anti-inflammatory bowel disease) and in clinical studies (eczema in infants and adults with irritable bowel syndrome) have been demonstrated.

6. Lactobacilli and Bifidobacteria as Interfering Agents against Quorum-Sensing

Up until 1970, the scientific community had established that bacterial growth and multiplication took place without communication between cells [102]. In the same year, *Photobacterium fischeri* was described as a new marine bacterial species able to produce a molecule that controlled both the luminescence and cellular density of the bacterial community [103]. Now, we know that this bacterium produces and releases signaling molecules called autoinducers (AI) that stimulate the bioluminescence in a population-density directed system. Approximately ten years later, the bioluminescence producing gene luminescence (lux) of *V. fischeri* and the AI of *P. fischeri* (N-(3-oxohexanoyl)-DL-homoserine molecule) were identified, leading to the presentation of a new fundamental concept, defined as the quorum sensing (QS) [104]. Initially strongly criticized, this theory stated that bacteria could communicate via small signaling molecules released to control growth; the entire activity is regulated by cell density in the community, which is able to finely tune the concentration of chemical signals [104]. More specifically, QS could be defined as a cellular communication mechanism used by bacteria to promote or repress a series of genes "beneficial" to the bacterium only if expressed by the whole community. The AI concentration drives bacterial information exchange through the action of quorum signals that accumulate within the bacterial environment. The QS system is based on a coordinated action between signaling molecules and sensor systems. Table 2 summarizes the main QS systems adopted by different bacterial species.

Table 2. Main pathways, signaling molecules and core proteins in QS system associated with the respective bacterial species.

Pathway	QS Signal Molecules	Core Proteins	Main Bacterial Species	References
luxslR	N-acyl-homoserine lactones (AHL)	LuxI, LuxR	*V. fischeri*	[105]
SmaI/SmaR		PhoR, PhoB	*Serratia* sp.	[106]
LasIR-RhlIR		Lasl, LasR, RhlI, RhlR	*P. aeruginosa*	[107]
Agr	Autoinducing peptides (AIP)	AgrA, AgrB, AgrC	*S. aureus*, *L. monocytogenes*	[108,109]
Extracellular protease processed AIP		plcR, OPP	*B. cereus*	[109]
Competitive quorum-sensing system		RapB,RapC, ComP, ComQ	*B. subtilis*	[107]
Cytolysin quorum-sensing system		CylA, CylB, CylM	*E. faecalis*	[110]
Fsr		FsrA,FsrB, FsrC	*S. aureus*, *E. faecalis*	[110,111]
LuxS/AI-2	autoinducer 2 (AI-2)	Pfs, LuxS	*V. harveyi*, *Haemophilus parasuis*, *Streptococcus agalactiae*	[112–114]
Lsr		LsrK, LsrR	*E. coli*	[115]

While Gram-negative bacteria produce acyl-homoserine lactone (AHL) (the earliest discovered prokaryotic signaling molecule) for intraspecific communication, Gram-positive bacteria synthesize unique autoinducing peptides (AIP) that differ from other bacteria in the form of precursor proteins (which undergo modifications during transport to become mature proteins). The LuxS/AI-2 system (LuxS/autoinducer-2) was initially described in *V. fischeri* but is now widely described in Gram-negative and Gram-positive bacteria and is known to allow intra- and inter-species exchange of signaling and communication. As a clarifying example, *E. coli* biofilms were shown to be susceptible to other signaling molecules produced by non-*E. coli* cells [116].

Since QS has evolved to control and modulate the gene expression of bacteria, microorganisms have naturally developed strategies to neutralize QS. Globally, these mechanisms are called quorum quenching (QQ) [117] and they inhibit the synthesis of virulence factors and communication through: (i) inhibition of signaling molecule generation [118,119], (ii) synthesis of structural analogues of signaling molecules which competitively bind with corresponding receptor proteins neutralizing the transmission of signal [120], and (iii) production of degradation enzymes which deactivate signal molecules [121].

Bifidobacterium is one of the most important probiotics in human health and possesses the LuxS/AI-2 QS systems, producing QS-signaling molecules including AI-2 and promoting biofilm formation [122]. Experimentally, the production of AI-2 in *Bifidobacterium* was positively improved up to 89.45% after adding carbohydrates [123]. The administration of *Bifidobacterium breve* to mice infected with Shiga toxin-producing *E. coli* (STEC) O157:H7 demonstrated strong anti-infective activity by the production of high concentrations of acetic acid (56 mM) inhibiting the expression of the Stx toxin of STEC [124]. The LuxS/AI-2 QS systems and the production of bacteriocin were also present in *L. plantarum*, a probiotic which controls the microecological balance of some important anatomical districts (e.g., intestine and vagina) and has practical applications in preserving food quality as well [125]. Some pathogens are susceptible to the *L. plantarum* QQ system (e.g., *P. aeruginosa* PAO1/ATCC 27853, methicillin resistant *S. aureus* ATCC 43300), which showed maximum activity against biofilm formation of *S. aureus* and pyocyanin production of *P. aeruginosa* [126]. Mouse models of burned skin were experimentally infected with *P. aeruginosa* and treated with the supernatant of *L. plantarum*. Results (after 5, 10, and 15-days post-infection) showed inhibition of *P. aeruginosa* colonization in the skin, liver, and spleen, suggesting the hypothesis that local probiotic administration had prevented the hematogenous dissemination of the pathogen [127]. In vivo studies have shown the

anti-streptococcal activity, against the oral pathogen *Streptococcus mutans*, of different probiotics (e.g., *Lacticaseibacillus casei* subsp. *casei* ATCC 393, *Limosilactobacillus reuteri* ATCC 23272, *L. plantarum* subsp. *plantarum* ATCC 14917, and *Ligilactobacillus salivarius* ATCC 11741) [87]. Hossain et al. (2021) developed a milk model to study the anti-listeria activity (against *L. monocytogenes*) of LAB by using *Lactobacillus sakei* and *L. plantarum*, which possess autoinducer-2 molecules [128].

The QS system might play a key role in the organization, formation, and maturation stages of the biofilms; hence, it could be regarded as an attractive target for the development of new antimicrobial agents. Indeed, the cascade of events controlled by QS is also sensitive to other factors, not only based on cell density but also to other environmental stimuli. The proper characterization of such internal regulators and external input remains a critical aspect in the development of strategies to contrast biofilm formation.

7. Strategies and Mechanisms Used by LAB to Fight Biofilms

The main critical stages that need to be tackled to successfully fight pathogenic biofilms are: (i) adhesion, (ii) maturation, and (iii) dispersion.

Lactobacilli are known to be effective in contrasting one or more of these steps in their action against pathogenic biofilms. Table 3 reports the most important molecular mechanisms exerted by LAB against most common human pathogens.

Table 3. This table reports a summary of the mechanisms of action used by some *Lactobacillus* and *Bifidobacterium* probiotics to contrast the establishment, growth, or stability of pathogenic bacteria.

Probiotics	Pathogens	Mechanism of Action	Study	References
Bifidobacterium animalis subsp. *lactis*	Periodontitis	Decrease of pro-inflammatory cytokine levels, blocked the recolonization of periodontal pockets.	CT	[129]
L. crispatus BCRC 14618, *L. pentosus*	*S. mutans*, cariogenic bacteria	Biofilm formation associated with sucrose-dependent cell-cell adhesion and the *gtfC* level of enzyme in the biofilm were decreased.	In vitro	[130]
L. fermentum, *L. paracasei*, *L. paracasei*, and *L. paracasei*	*S. mutans*	Decreased *S. mutans* biofilms.	In vitro	[131]
L. salivarius strains	*S. mutans*	Reduced bacterial growth and expression levels of *gtfB*, *gtfC*, and *gtfD gtfs* as well as EPS production.	In vitro	[132]
L. salivarius	*S. mutans* with *C. albicans*	Secretory factors inhibited the formation of biofilm and fungal morphological transformation, with reduction of *C. albicans* pathogenicity.	In vitro	[133]
L. fermentum 20.4, *L. paracasei* 28.4, *and* *L. rhamnosus* 5.2	*C. albicans*	Reduced expression levels of *ALS3*, *HWP1*, *CPH1*, and *EFG1*.	In vitro	[134]
L. rhamnosus GR-1 *and* *L. reuteri* RC-14	*C. glabrata*	Reduced expression of biofilm-related genes (*EPA6* and *YAK1*).	In vitro	[135]
B.bifidum, *L. acidophilus*, *L. brevis*, *L. casei*, *and* *L. rhamnosus* GG	*S. mutans*	Reduced expression of *gtfs* and glucan.	In vitro	[136]
L. casei Shirota, *L. casei* LC01, *L. plantarum* ST-III, and *L. paracasei* LPC37	*S. mutans* strains, multispecies biofilms	Prevention of *S. mutans* and multispecies biofilms growth.	In vitro	[137]
L. kefiranofaciens, *L. plantarum*, *L. rhamnosus*, *L. johnsonii*	*S. mutans*, *S. sobrinus*	Shutdown of all biofilm-associated genes encoding carbohydrate metabolism, regulatory biofilm, and adhesion proteins.	Na	[138]

Table 3. *Cont.*

Probiotics	Pathogens	Mechanism of Action	Study	References
L. casei, *L.reuteri*, *L. plantarum*, *L. salivarius*	*S. mutans*	Decrease in expression of genes involved in acid tolerance, QS and EPS production. *L. salivarius* had peroxide-dependent antimicrobial and antibiofilm activities.	Na	[87]
Combinations of *L. plantarum*, *L. helveticus*, *and S. salivarius*	*C. albicans*	Reduced expression of *EFG1*, *HWP1*, *ALS3*, and *SAP5*.	Na	[139]
L. gasseri and *L. rhamnosus* supernatant	*C. tropicalis*, *C. krusei* and *C. parapsilosis*	Disruption of mature biofilm, inhibition of mixed biofilms, and cell damages on silicone surface.	In vitro	[140]
L. pentosus strain LAP1	*C. albicans*, *C. tropicalis*, and *C. krusei.*	Antibiofilm property.	In vitro	[139]
L. casei LBl	*S. aureus* strains 9P and 29P	Biosurfactants dispersed the preformed biofilms.	In vitro	[141]
L. acidophilus ATCC 4356	*B. subtilis* BM19	Growth and biofilm formation inhibition.	Na	[142]
L. plantarum F-10 supernatant	*P. aeruginosa* PAO1, MRSA and hospital-derived strains	Downregulation of QS signals, oxidative stress in wound healing stages, inhibition of the virulence factors (motility, activity of protease and elastase, production of pyocyanin and rhamnolipid).	Na	[126]
EPS-Lp from *L. plantarum* and EPS-B from *Bacillus* spp.	*E. coli* ATCC 35218	EPSs reduced cell surface hydrophobicity level, indole production, prevented biofilm formation, reduced efflux pumps devoted to bacterial adhesion and antimicrobial resistance.	Na	[143]
L. fermentum TCUESC01 and *L. plantarum* TCUESC02	*S. aureus*	Biofilm formation inhibition by alteration of the ica operon (*icaA* and *icaR*).	Na	[83]
L. fermentum (KT998657) isolated from neonatal fecal samples	*P. aeruginosa* PAO1	Reduced biofilm forming due to postbiotics (bacteriocin and EPS), bacteriocins creates pores in the cell membrane resulting in cell death. Alteration of matrix and cell assembly, cell-cell interaction and attachment to form biofilms.	Na	[144]

Abbreviations: CT = clinical trial; Na = not available

One of the easiest mechanisms to contrast the growing of pathogen is niche occupation and resident bacteria displacement. LAB can also produce molecules able to contrast biofilm formation even without the presence of bacterial cells.

Culture supernatant (CS) of isolates of *Lactobacillus* spp. was shown to inhibit the biofilm formation of *V. cholerae* by more than 90% compared to controls. CS (pH neutralized) eliminated the antimicrobial activities of lactobacilli against *V. cholerae* but had negligible effects on their biofilm inhibitory potential. Furthermore, CS of all the lactobacilli isolates caused the dispersion of preformed *V. cholerae* biofilms in the range of 62–85%; nevertheless, pH neutralization of the CS reduced the biofilm dispersal potential of some isolates. Curiously, the study showed that CS of none of the lactobacilli isolates had antimicrobial activity against *V. parahaemolyticus*, but many of them inhibited the formation of its biofilm. However, none of the CSs dispersed the preformed biofilms of *V. parahaemolyticus*. The ability of CS to inhibit the adherence of *Vibrio* spp. to the epithelial cell line was also determined. The study concluded that the biofilm dispersive action of CS of lactobacilli is strain-specific and pH-dependent. As *Vibrio* spp. is known to form biofilms in the intestinal

niche having physiological pH (range 6–7), the probiotic strains that have dispersive action at high pH may have better therapeutic potential [90].

Bacteriocins are a class of antimicrobial peptides, which are synthetized in ribosomes and are often more potent than their antibiotic counterparts [145]. Overall, a deeper understanding of the precise means by which a biofilm forms on a substrate as well as insights into the mechanisms by which bacteriocins inhibit biofilms require further investigation; this is probably the reason behind a wide application in the food industry but a still limited application in medical settings. However, bacteriocins, in particular those produced by LAB, exhibit relatively low levels of cytotoxicity towards human and animal tissues. Indeed, the non-toxic nature of nisin, one of the most famous and widely used bacteriocins, has been highlighted in a number of studies [146,147].

8. Discussion

The biofilm represents a biological, highly organized, three-dimensional system where the bacteria are structured into a functional community, which can be formed by single or multiple species [148]. Biofilms are constituted by sessile bacteria, genetically identical to their planktonic counterparts, embedded in an EPS matrix, produced by the same bacteria. More specifically, the sessile–planktonic transition is characterized by profound physiological changes, induced by environmental and genetically controlled stimuli [149,150]. Recent studies suggest that biofilm transition could be triggered by a regulation cascade in which transcription regulators might have a relevant role. One of such key factors has been identified in the protein CcpA (catabolite control protein A), for which a role in the regulation of the central metabolism of carbon in low GC Gram-positive bacteria has been described [151,152]. CcpA is only one of the many transcription regulators, described over the years, that has been shown to be involved in biofilm formation in several bacterial species [153,154]. Other mechanisms and pathways that likely play an important role in biofilm formation are: cAMP-CRP-regulated pathways, c-di-GMP-dependent polysaccharides biosynthesis, and the GacS/GacA two-component regulatory system as a super-regulator of QS, as widely discussed in this review [155–157].

Compared to motile cells in the planktonic state, once the bacteria are embedded into the biofilm, they are inherently less susceptible to antimicrobial agents; hence, they are more resistant to eradication. Therefore, pathogenic biofilms remain one of the main obstacles that need to be overcome for a successful drug-resistant bacterial elimination.

The large portion of clinically important pathogenic and opportunistic bacteria (e.g., MRSA, *S. epidermidis*, *P. aeruginosa*, *Gardnerella vaginalis*, and *S. mutans*), responsible for difficult-to-eradicate infections in nosocomial settings, are able to form biofilms and have been under focus for increasing antibiotic resistance. Such biofilm-forming MDR bacteria represent a serious menace to public health. One of the most effective ways to fight biofilms consists of attempting to disrupt the initial steps of biofilm formation, including the adhesion and aggregation of bacteria. Intervening in an early phase has the additional advantage of requiring a lower concentration of inhibiting molecules, compared to removing a fully established and stable biofilm.

LAB are beneficial bacteria that have shown a marked utility in preventing and treating gut, oral, and urinary infections. LAB protect the host by different mechanisms, such as decreasing pH, producing antimicrobials, providing competitive exclusion of pathogens, and reducing excessive inflammation [33]. LAB, upon adhesion on a solid surface, form robust biofilms as well, and such bacterial aggregative forms are not structurally different from their pathogenic counterparts. However, the host response is not triggered and activated as in the case of pathogenic biofilms. In vivo, LAB biofilms are part of the microbiome found in the gut and vagina, and their absence can be detrimental to the host.

The EPS matrix protects the bacteria from the action of the immune system, representing a physical barrier to antibodies and phagocytes as well. Indeed, it is the protective EPS slime alone that confers a consistent part of the medium-to-high levels of resistance to antibiotics and disinfectants [158].

Different chemical, biochemical, and enzymatic products that disrupt the EPS matrix in biofilms have been used for long time; however, many of them are starting to become ineffective [159].

Among the EPS-degrading enzymes, glycoside hydrolases are rather successful, and they have been shown to inhibit pre-existing bacterial biofilms as well. Their efficacy can be altered by environmental conditions; however, by interfering with the stability of biofilms they can potentiate the impact of antibiotics [160]. DispersinB causes cleavage in the EPS matrix (targeting linear polymers of N-acetyl glucosamine), damaging biofilms of several species of bacteria, while nucleases (DNase) can also disrupt the stability of the matrix by hydrolysing eDNA [161,162]. Such enzyme types were shown to detach pre-attached *S. aureus* and *S. epidermidis* biofilms as well [163].

Innovative strategies, aimed at disassembling the EPS matrix of bacterial biofilms, have been investigating novel disruptive agents, nanoparticles, and technologies, such as the application of magnetic fields, photodynamic therapy, and ultrasounds, with the synergistic effect of antibiotics [164].

In recent decades, the discovery of new types of antibiotics has been limited, while the emergence of resistance amongst pathogens with a propensity for biofilm formation is on the rise; therefore, solutions are urgently required. Thus, the development of novel therapies and approaches to fight pathogenic biofilms must remain an active field of research. The focus is currently on alternative and, perhaps, underestimated therapeutic agents able to prevent biofilm formation and/or disaggregate and disperse already established biofilms.

One of the promising strategies in biofilm treatment is represented by bacteriophages and their lytic proteins able to kill bacteria [165]. Accumulating evidence is highly supportive on the utility of phages in contrasting biofilm formation in catheters and prosthetic infections, and in limiting biofilm growth on human tissues [166]. A broad application in humans is hindered by technical limitations in the production of high-quality and purified phages, and concern for their capacity to transmit toxin or resistance genes among bacteria.

Fecal microbiota transplantation (FMT) and probiotic administration are the two major strategies to change the composition of the gut microbiome; however, the development of therapeutic application has met substantial challenges. While the first procedure can present some risks in terms of opportunistic bacterial transmission, the use of probiotics might have the advantages of being considered safer and cheaper. In some cases, the oral administration of probiotics can be ineffective because non-endemic bacteria can face challenges in properly colonizing hostile surfaces, such as the human intestines. Probiotics and their capacity to regulate the immune system have long been considered a potential anti-tumor strategy. In a recent study, smectite, a type of mineral clay and established anti-diarrhea drug, has been tested for the capacity to enhance probiotics expansion (especially *Lactobacillus* spp.) in the murine gut and to elicit anti-tumor immune responses [167]. The ion-exchangeable micro-structure of smectite preferentially promotes LAB to form biofilms in vitro and in vivo. In mouse models, smectite loaded with LAB biofilms (*Lactobacillus* and *Bifidobacterium*) inhibited tumor growth (when used alone) and enhanced the efficacy of chemotherapy or immunotherapy (when used in combination with either of them); the mechanism of action could be based on the activation of dendritic cells (DCs) via Toll-like receptor 2 (TLR2) signaling.

In the last decade, among the different methods for biofilm control, even novel bio-engineering strategies have been considered. Bacteria inside biofilms can be more easily eliminated by conventional antibiotics after the biofilm structure has been perturbed by ultrasound or electric fields.

In nature, many sessile organisms are able to produce chemicals capable of interfering with biofilm formation, and some of these substances have been used at an industrial level to control the growth of biofilms.

In addition to the most widely recognized benefits provided by microbial biofilms, novel "useful" services, such as biodegradation of toxic compounds and pollutants, biore-

mediation, and toxic effluents treatment, have been recently described [168,169]. These applications suggest that microbial biofilms could be successfully used for new applications in the biomedical, industrial, food, and environmental field. In human medicine, their ability to colonize hostile niches and outcompete pathogens may significantly contribute to host health.

While LAB biofilms are known to contrast the growth of pathogenic microorganisms, the network of interactions with other LAB members or other beneficial bacteria in vivo are difficult to investigate. We can expect that quorum sensing (QS) plays a crucial role in multispecies biofilm formation and in the stability of a healthy microbial community [102]. LAB are known to create positive interaction among them, as supported by evidence in laboratory co-cultures [170,171]. While competition or negative interactions among LAB and beneficial or commensal members of the microbiota in vivo cannot be excluded, LAB probiotic biofilms in the healthy gut or in other human niches are expected to promote cooperation and microbiota stability, enhancing the contrasting effects to pathogen colonization, and favoring the exchange of nutrients between the host and the microbiota.

In conclusion, the progresses made in the field of probiotics are still growing slowly due to the numerous novel bacteria discovered every year, and because of the lack of well-conducted, independent clinical trials since, given the diversity of probiotic candidates, they are too often considered all equally potent and therefore inadequately investigated at the species or even strain level.

9. Conclusions

Specific probiotic combinations are demonstrating day-by-day to have a marked utility in the human field, and data on antibiofilm activity on various respiratory, genito-urinary, wound, and tissue pathogens, are starting to become convincing. However, there is still a long way to go, especially in their in vivo routine usage. This review should encourage better investigation on probiotic–biofilm interactions and how to fight biofilm infections through the so-called "good bacteria", such as bifidobacteria and lactobacilli, highlighting that there are "useful" or "good" biofilms as well. Mechanisms of action and antibiofilm activities must be considered as strain-related; therefore, we will need to focus our research on the development of such promising strains. It is often debated whether probiotics will become broadly used drugs or medicaments in the future; it is still too early to say, but given the uncertain longevity of antibiotics, it would be recommended to explore alternative means, and so far, probiotics represent one of the most promising.

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