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Can Probiotics Emerge as Effective Therapeutic Agents in Apical Periodontitis? A Review

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Abstract

Apical periodontitis (AP) is a biofilm-associated disease initiated by the invasion of dental pulp by microorganisms from the oral cavity. Eradication of intracanal microbial infection is an important goal of endodontic treatment, and this is typically accomplished by mechanical instrumentation and application of sodium hypochlorite and chlorhexidine. However, these agents are tissue-irritating at higher concentrations and cytotoxic. Certain probiotics have been found effective in controlling marginal periodontitis, as evidenced by reduction of pathogenic bacterial loads, gains in clinical attachment levels, and reduced bleeding on probing. In vitro studies have shown inhibitory activity of some probiotics against endodontic pathogens. Similarly, in vivo studies in rats have demonstrated a positive immuno-modulatory role of probiotics in AP, as manifested by decreased levels of proinflammatory markers and increased levels of anti-inflammatory markers. A role for probiotics in effecting a reduction of bone resorption has also been reported. This review provides an outline of current research into the probiotic management of AP, with a focus on understanding the mechanisms of their direct antagonistic activity against target pathogens and of their beneficial modulation of the immune system.

Keywords Apical periodontitis · Probiotics · Root canal treatment · Pathogens · *Enterococcus faecalis* · *Candida albicans*

Introduction

Apical periodontitis (AP) is inflammation of the periradicular tissue initiated by the microbial invasion of the tooth pulp [1–3]. The clinical presentation varies between classical signs of inflammation such as pain, swelling, and loss of function to a complete absence of discomfort [2]. A recent review article by Jakovljevic et al. [4] reported the worldwide prevalence of AP as 6.3%, an overall increase of around 1% since the last study in 2012 [5]. Similarly, the prevalence of AP in endodontically treated teeth increased from 35.9 to 41.3%. This increased prevalence of AP is a cause of concern as asymptomatic AP can go undiagnosed for a long time and may significantly add to the overall inflammatory burden in the body. Various inflammatory markers found elevated in root canal infections are also associated with other systemic inflammatory conditions [6]. The prospect of an etiological association of cardiovascular

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disease with AP has generated interest within the scientific community following the reporting of signs of endothelial dysfunction in patients experiencing AP [7, 8]. AP-induced inflammatory mediators, along with activated immune cells, may also play a role in the development of insulin resistance and diabetes mellitus [9]. In other studies, the presence of periapical lesions has also been linked to poorer metabolic control in type 2 diabetic patients [10] and to unfavorable birth outcomes in pregnant females [11].

Root canal treatment (RCT) is performed for the management of AP and involves the elimination of microbes and their toxic products from the root canal of the tooth, followed by filling with an inert material [12]. This treatment also limits the spread of infections to the jaw and the other parts of the body [13]. The current protocols for root canal disinfection are directed at the non-specific killing of microbes by the combination of mechanical instrumentation and use of irrigants and medicaments such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), and calcium hydroxide (CH) [14, 15]. Although root canal treatment achieves a higher success rate of around 85–95% [16], demographic studies have reported the prevalence of post-treatment AP to be in the range of 21–65% [13]. Most endodontic treatment failures are attributed to persistence of intracanal infection [17, 18]. Factors contributing to this are the complexity of root canal morphology, which hinders mechanical cleaning processes [19] and the intractable nature of many of the infecting microbial species when organized in the form of a biofilm [20]. Biofilm-inhabiting microbes are known to be considerably less sensitive to various stressors than they are when in planktonic communities [21]. Endodontists continue to actively seek new treatments to increase the efficiency of root canal biofilm elimination and to provide them with more predictable and successful treatment outcomes for AP [22].

Probiotics have evolved as a treatment alternative of considerable interest owing to their reported beneficial effects in managing chronic inflammatory conditions of the gut [23]. Also, a potential therapeutic role of probiotics in treating psychiatric disorders such as anxiety, depression, and autism through the gut-brain axis by modulating the gut microbiota has attracted attention in recent times. The gut-brain axis has as its basis the premise that the gut microbiota can influence brain functions and vice versa. This interaction is regulated by neural, endocrinal, and immunological components and is considered to have an important role in maintaining homeostasis [24, 25]. In addition, probiotics may have a beneficial role in relieving the stress symptoms of irritable bowel syndrome and chronic fatigue syndrome. In other studies, probiotics have been implicated in delaying the progression of Alzheimer's disease in a mouse model owing to their ability to restore cerebral glucose homeostasis [26]. Much like the gastrointestinal tract activities of probiotic lactobacilli, which inhibit colonization of the gut by pathogenic bacteria [27], certain probiotics may prevent growth of and biofilm formation by the disease-causing bacteria in the oral cavity [28]. Furthermore, probiotic-mediated modulation of the immune response may also play an important role in limiting the extent and severity of chronic oral diseases [29]. More specifically, beneficial outcomes associated with the use of probiotics have been reported in clinical studies of dental caries [30, 31], gingivitis [32], and marginal periodontitis [33, 34]. However, studies exploring the potential role of probiotics in the treatment of AP are relatively scarce and are largely limited to laboratory-based and animal studies. Certain probiotic *Lactobacillus* and *Bifidobacterium* strains exhibit in vitro antimicrobial activity against the important endodontic pathogens, *Enterococcus faecalis* and *Candida albicans* [35], and anti-inflammatory activities have also been reported in a Wistar rat study [29]. Moreover, it is important to note that to date, in these reported studies, there has been considerable methodological heterogeneity in the study designs and in the selection criteria for the probiotics as well for their dosing schedules and because of this it is difficult to make generalizations about the significance of the clinical outcomes. Nevertheless, probiotics appear to provide a novel and very promising therapeutic option, especially considering the stark limitations of the currently available conventional treatment regimens and their role in the management of AP should continue to be explored using carefully standardized study designs. This review critically explores the prospects for application of probiotics in the management of AP.

Etiology and Pathogenesis of Apical Periodontitis

Mixed consortia of Gram-negative bacteria such as *Fusobacterium nucleatum*, *Prevotella intermedia*, *Treponema denticola*, *Tanneralla forsythia* and *Porphyromonas gingivalis*, and the Gram-positive *Ent. faecalis* and various members of the genera *Parvimonas, Filifactor*, *Pseudoramibacter*, and *Streptococcus* are encountered amongst the most prominent endodontic pathogens [36, 37]. Moreover, it has been estimated that more than 50% of endodontic bacteria remain uncultivated [38]. This highlights the considerable lacunae that exist concerning the exact role of many bacterial species in AP and their contributions to failures in treatment outcomes [20]. The unique attributes of biofilm-associated microbial communities have emerged as central to our understanding of many infectious diseases including root canal infections. Biofilms of microorganisms tenaciously attached to root canal surfaces are frequently found associated with apical

periodontitis [20, 39]. The potential significance in AP of microbial biofilms on root canal walls along with amorphous material filling the intercellular space was first reported by Nair [40]. Scanning electron microscopy studies were soon visualizing bacterial colonization of the root tip affected by AP [41]. In a histopathological study, the presence of bacterial biofilms was visualized in both untreated and treated canals, the prevalence however being more in teeth having larger radiographic lesions [42]. Preventing biofilm formation and its elimination if present are of prime importance for the achieving of root canal disinfection [37]. Microbes enjoy numerous benefits when present in biofilm communities as compared with when they are adopting a planktonic lifestyle. Of particular significance are the facilitation of gene transfer (including antibiotic resistance determinants), the reduced susceptibility to antimicrobials, and the increased protection against the host immune response [21, 43]. All of these factors contribute to make complete elimination of root canal infection extremely difficult to achieve [37].

The essential role of microbes and the host response to their presence reveals AP as a pathologically dynamic condition [44]. Once microorganisms invade a root canal, their toxins, and bioactive metabolic products accumulate in the periapical area inducing a host-mediated inflammatory response. The microbial toxins have pathogen-associated molecular patterns, and these can be identified by pattern recognition receptors including toll-like receptors (TLR) [45, 46]. TLR intercept various microbial components and initiate inflammatory cascades releasing numerous proinflammatory cytokines and inducing neutrophil phagocytosis. Adaptive immunity is activated by dendritic cells (DC) as via antigen processing and presentation to T cells [47]. Periodontal tissues contain two types of immature DC: Langerhans-type DC ($CD1a^+$) and interstitial type DC ($CD1a^-$). These identify and process antigens, following which they then migrate to lymph nodes and stimulate T-cell immune responses [48]. While the host immune system endeavors by such mechanisms to eliminate microbial infection, microorganisms when present in biofilms can camouflage their immunogenicity and evade host immune reactions [49]. Thus, the ultimate outcome of incipient AP is largely dependent upon the dynamics of this classical tussle between microbe and host. The bone resorption in AP is a dynamic process and is governed by osteoblast-osteoclast interactions [50]. The triad of RANK (receptor activator of nuclear factor kappa-B), RANKL (RANK ligand), and OPG (osteoprotegerin) are involved in signaling for bone formation-resorption processes [51]. RANK is produced on osteoclast precursor cells, and RANKL is expressed by osteoblastic cells. Binding of RANK to RANKL leads to differentiation and activation of osteoclasts, resulting in increased bone resorption. OPG, on the other hand, blocks this interaction by binding with RANKL leaving unattached RANK [50]. Endodontic pathogens promote bone resorption either directly by stimulating RANKL expression or by stimulating the production of proinflammatory mediators like TNF-α and IL-1β [51].

Disinfection Strategies in Root Canal Treatment: Past, Present, and Future

The ability to clean and shape root canals is critical, as bacteria remaining after treatment are the most important predisposing factors causing post-treatment AP. Various studies have found reduced microbial counts effected by mechanical instrumentation, even without application of an irrigant [52]. However, accessibility to mechanical cleaning especially in recondite parts of root canals remains a challenge [53]. To complement mechanical instrumentation, antimicrobial irrigants are used. This serves multiple functions including disinfection of the entire root canal, flushing out of debris including bacterial cells and their metabolites, organic and inorganic tissues, lubrication of the root canal, and inactivation of endotoxins [54]. NaOCl is a commonly used antimicrobial irrigant for root canal disinfection. Interestingly, it is still delivered in concentrations of between 0.5 and 6%, and there is an ongoing debate regarding the ideal concentration for intracanal irrigation. A recent study reported no difference between the use of low and high concentrations of NaOCl and subsequent healing of AP [55]. Also, no difference in the number of postoperative samples having cultivable bacteria was reported [56]. The bis-biguanides (especially CHX) are also commonly used endodontic irrigants. The positively charged CHX molecule interacts with the negatively charged bacterial cell wall causing structural damage and even cell death when used at high concentrations [57]. Bacterial survivors were found however after irrigation with either NaOCl or CHX in a clinical study of persistent root canal infections [58]. Although the use of NaOCl has a long history of safe and successful use within the root canal space, its extrusion into the periapical area, as evidenced by profuse bleeding, acute pain, and immediate swelling, may be associated with an array of cytotoxic effects ranging from ulceration, hemolysis, and necrosis to damage to the nerves [59, 60]. Reports of the cytotoxicity of CHX when used in higher concentrations also underline the potential risks associated with its application to human tissues [61]. These studies indicate the need for improvement in the application protocols of existing irrigants. Substitution or addition of newer and more effective irrigants is required to address the complex problem of persistent root canal infection. Novel disinfectants such as ozone [62, 63], photodynamic therapy [64], nanoparticles [65], and cold atmospheric plasma [66,

67] have been tried with variable outcomes and only limited clinical success in the field of endodontics.

Probiotics in Oral Health

According to the Food and Agricultural Organization and World Health Organization, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [68]. The field of probiotics has rapidly developed owing to the combination of improved knowledge, technology, and consumer awareness [69]. While health promoting strains are reported for many species, this review is focused on lactic acid bacteria (LAB) and the genus *Bifidobacterium* because of their role in the health of the oral cavity. A variety of strains of LAB, particularly members of the genus *Lactobacillus*, are now most commonly used as probiotics [70], for food preservation [71, 72] and as therapeutic agents [73]. Some LAB have also been used to help control certain metabolic syndromes, cancer, and obesity [74]. The LAB are ubiquitous and are found in a diverse range of natural habitats including plants, animals, manures, and a variety of human tissues including the gastrointestinal tract, oral cavity and vagina [75, 76]. They are all Gram-positive, non-spore-forming, catalase-negative, and non-motile bacteria. *Bifidobacterium* species such as *B. bifidum*, *B. animalis*, and *B. breve*, are naturally occurring residents of the human gut and reportedly provide numerous benefits to their human host, ranging from protection against pathogenic bacteria such as *Helicobacter pylori*, cancer and aberrant activation of the immune system as well as helping to control serum cholesterol levels, improving lactose intolerance [77] and reducing host susceptibility to infection and allergies [78].

The beneficial effects of certain probiotics in modulating the gut microbiota and improving gastrointestinal health are now well recognized [79, 80]. The functional similarities between the biofilms of the gut and of the oral cavity have subsequently led to the introduction of oral cavity probiotics for the management of chronic dental diseases such as dental caries and marginal periodontitis [81]. Both of these conditions are multi-factorial in their pathogenesis, but their underlying etiologies are linked to multi-species biofilms. However, acidogenic bacteria such as *Streptococcus mutans* and *Lactobacillus* in the presence of favorable environmental conditions cause caries [82]. On the other hand, proteolytic bacteria such as *P. gingivalis*, *T. forsythia*, and *T. denticola* are strongly linked to the development of periodontitis [83]. Dental caries is considered to be the most common disease affecting humans and is characterized by the dissolution of minerals and the destruction of the organic content of the teeth [84]. Probiotics have increasingly been investigated as a prophylactic measure for the prevention of dental caries [85]. Indeed, various formulations of probiotic strains of *Lact. rhamnosus* [86, 87], *Lact. reuteri* [88] and *Lact. paracasei* [89], and *Bifidobacterium animalis* subsp. *lactis* [90] have been reported to have anti-caries activity. The observed reduction in caries activity associated with their use has been attributed to their activity in preventing biofilm formation and in interfering with streptococcal colonization [29, 87, 91].

Periodontitis, with a reported prevalence of around 50%, is manifested as inflammation of the tooth-supporting tissues and it occurs in response to tissue damage elicited by biofilm-located pathogens [92]. *Lact. salivarius* [33], *Lact. reuteri* [93], *Lact. casei* [94], and *B. animalis* [95] have been used with some success to control marginal periodontitis. Few naturally occurring species of lactobacilli in the oral cavity have been shown in vitro to exhibit antimicrobial activity against common periodontal pathogens [75]. Multi-dimensional activities of probiotics involving both the direct inhibition of pathogen growth and the beneficial modification of the host tissue response by reducing formation of proinflammatory molecules and stimulating the output of cytoprotective proteins are considered to be the basis for the observed beneficial outcomes associated with the use of probiotics [96]. A systematic review of 12 randomized controlled trials and three review articles concluded that oral probiotics have a beneficial role in maintaining oral health by reducing the counts of "oral pathogens" [97]. Another meta-analysis reported significant clinical attachment gains associated with the adjunctive use of probiotics in chronic periodontitis patients [98]. In other studies, the auxiliary use of probiotic *Lact. paracasei* in milk led to significantly less gingival inflammation [32] and *Weissella cibaria* was reported to inhibit *Fusobacterium nucleatum* and to reduce the production of some of the volatile sulphur compounds responsible for halitosis [99]. *Streptococcus salivarius* K12 has antimicrobial activity against bacteria involved in halitosis [100]. The potential role of bacilli as probiotics for oral health maintenance has also been evaluated, albeit with mixed results. *Bacillus subtilis*-containing mouth rinses [101] and tablets [102] were reported to have a beneficial role in treatment of periodontitis, reducing the number of periodontal pathogens and improving clinical parameters such as bleeding on probing and probing depth. However, in another double-blind placebo controlled randomized controlled trial *B. subtilis, B. megaterium*, and *B. pumilus* had no influence on gingival indices and bleeding on probing [103]. It was consistently observed that the health benefits of the probiotics were strictly dependent on the strain type, dose and duration of application.

It should be mentioned that dental caries has a direct contributory role in the etiology of AP. The demineralized

tooth structure caused by caries provides an easy portal for bacterial entry to the dental pulp, where they can cause pulp necrosis and subsequently AP [44]. While the role of marginal periodontitis in causing pulpal changes is controversial [104], a beneficial effect of periodontal therapy on endodontic outcomes has been reported [105, 106]. Therefore, understanding the efficacy of probiotics in these conditions may help in formulating and selecting probiotic strains having the dual roles of prevention and therapy in the management of AP.

Probiotics in Apical Periodontitis

Five laboratory and two animal studies assessing the potential beneficial role of *Lactobacillus* species in the treatment of AP (one in combination with a *Bifidobacterium* strain) are depicted in Table 1. Laboratory-based studies examined the antimicrobial effect of cell-free supernatants (CFS) [35, 107, 108] and lipoteichoic acid extracts from *Lact. plantarum* [109, 110]. Strains from a commercial probiotic cocktail significantly inhibited the growth of planktonic and biofilm preparations of *Ent. faecalis* and planktonic *C. albicans* [107]. In another study, unconfirmed *Lact. rhamnosus* and *Lact. plantarum* displayed strong activity against *Ent. faecalis* [108]. *Lact. plantarum* ATCC 8014 and *Lact. rhamnosus* ATCC 7469 were also strongly inhibitory to *Ent. faecalis* and *C. albicans* in both planktonic and biofilm cultures; however, *B. bifidum* ATCC 11863 appeared only to be inhibitory to planktonic bacteria [35]. Systemic administration of the two probiotics, *Lact. rhamnosus* LR04 and *Lact. acidophilus* LA14 significantly reduced the bacterial counts in both the saliva and root canals of rats [29]. In another set of in vitro studies, lipoteichoic acid from *Lact. plantarum* displayed antimicrobial activity in both a multi-species biofilm comprising *Actinomyces naeslundii*, *Ent. faecalis*, *Lact.salivarius*, and *Strep. mutans* [109], and in a single-species biofilm of *Ent. faecalis* [110]. Lipoteichoic acid is a biologically-active cell wall component of Gram-positive bacteria such as *Lactobacillus* species that has been found to inhibit *Ent. faecalis* biofilms in laboratory studies [111]. *Lact. plantarum* lipoteichoic acid (Lp.LTA) has been reported to inhibit biofilm formation by certain oral cavity pathogens, such as the cariogenic species *Strep. mutans* via disruption of quorum sensing processes [111]. Lp.LTA can also exhibit a favorable immunomodulatory role by enhancing the production of anti-inflammatory cytokines. Recently, the term postbiotics has been coined to encompass biologically active bacterial components such as lipoteichoic acid that can function to help "support health and/or well-being" [112].

Oral supplements of *Lact. rhamnosus* LR04 and *Lact. acidophilus* LA14 have been assessed in animal models [29, 113]. Lower levels of the proinflammatory markers IL-1β and IL-6 and a higher level of the anti-inflammatory marker IL-10 were reported following 30 days of probiotic supplementation in rats [29]. Follow-up studies using the same probiotics and similar methodology demonstrated a reduction in RANKL (receptor activator of nuclear factor kappa-B ligand) and TRAP (tartrate-resistant acid phosphate) levels, and raised OPG (osteoprotegerin) levels, together with a significantly lower volume of bone resorption in the probiotic treatment group by comparison with the control group. However, no significant difference in plasma calcium and phosphorous levels were reported [113]. These studies are supportive of probiotics having a potential therapeutic role in AP due to their antimicrobial, anti-biofilm, anti-inflammatory, and immuno-modulatory effects and also their potential role in fostering bone resorption and in preventing secondary infections.

Antimicrobial and Antibiofilm Activity

Inhibition of pathogenic microorganisms has been considered one of the important properties of probiotic LAB. The antimicrobial activity of these probiotics is due to the production of lactic acid, hydrogen peroxide, and bacteriocins. Bacteriocins are antimicrobial peptides which are generally most strongly inhibitory to phylogenetically related strains. These antimicrobials are regarded as safe due to their low toxicity and their relatively narrow inhibitory spectra against other competitor bacteria [114, 115]. Antimicrobial activity of specifically selected probiotics has been detected against *Ent. faecalis* and various other oral pathogens including the fungus *C. albicans* in both planktonic and biofilm assays [104]. Probiotics can interfere with the growth of other microbes (including potential pathogens) by various mechanisms including competition for nutrients and space. Hydrogen peroxide can cause cell damage to and ultimately kill susceptible microorganisms. Commercially available formulations containing *Strep. salivarius* K12 and *Strep. salivarius* M18, both of which were originally derived from the oral microbiotas of healthy human subjects, have been shown to modulate the oral microbiome through their production of potent bacteriocin activity against several oral cavity pathogens including *Strep. pyogenes* [116] and *Strep. mutans* [117]. In other studies, reuterin (produced by *Lact. reuteri*) has been shown to exhibit antimicrobial activity against *Strep. mutans* and *P. gingivalis* [88, 118].

Anti-biofilm activity of probiotics has been detected in various in vitro model studies of dental caries [119]. The action of probiotics against pathogenic bacteria in biofilms is both multifaceted and complex [27] and varies according to the effector probiotic strain utilized [120].

Table 1 Applications of probiotics and their metabolites in apical periodontitis

Probiotics	Methods	Results	References
Lact. rhamnosus LR 04 and *Lact. acidophilus* LA 14	30-day oral supplements in male Wistar rats after induction of AP in mandibular molars Blood, micro-computed tomography and immunohistochemical analysis was done	Alkaline phosphatase levels were significantly higher in probiotic groups (332.3, 322.6, and 234.8 U/L in *Lact. rhamnosus*, *Lact. acidophilus*, and control groups, respectively). No significant difference in plasma calcium and phosphorous levels was reported Micro-computed tomography analysis revealed significantly lower volume of bone reduction in the probiotic groups than the control group Probiotic groups had significantly lower levels of RANKL and TRAP compared with the control group upon immunohistochemical analysis. OPG level was significantly more in *Lact. acidophilus* than *Lact. rhamnosus* and control groups	[113]
Lipoteichoic acid purified from *Lact. plantarum*	Effect of lipoteichoic acid assessed against *Ent. faecalis* ATCC 29212 cultured in glass bottom dishes and human dentin blocks for 3 weeks Confocal laser scanning and scanning electron microscopy was performed	Biofilm formation and disruption increased with lipoteichoic acid application in a dose dependent manner Antimicrobial action of medicaments like CHX and CH was enhanced after lipoteichoic acid application	[110]
Lact. rhamnosus LR 04 and *Lact. acidophilus* LA 14	24 male Wistar rats were divided in three equal groups and AP was induced in the maxillary molars followed by oral probiotic supplements in two test groups and one control group Blood, microbiological, histopathological and immunohistochemical analysis was done	No significant differences in blood counts between the probiotics and control group Significantly lower CFU in saliva 7.6, 8.0 and 8.7 and in root canal 8.0, 8.4 and 9.07 in *Lact. rhamnosus*, *Lact. acidophilus* and control group respectively Periapical lesion size in control group was 47.72 and 28.62 and 34.93×10^4 μm^2 in *Lact. rhamnosus* and *Lact. acidophilus*, respectively. The difference was significant between the probiotics and control group Immunohistochemistry analysis revealed significantly lower levels of proinflammatory cytokines (IL-6 and IL-1) and higher levels of anti-inflammatory cytokine (IL-10) in the probiotic groups compared with control group	[129
Purified lipoteichoic acid from *Lact. plantarum*	Multi-species biofilm comprising *Actinomyces naeslundii*, *Ent. faecalis*, *Lact. salivarius*, and *Strep. mutans* was cultured anaerobically in PYG media on glass plates and on human dentin slices Confocal laser scanning microscopic, crystal violet assay, and scanning electron microscopic analysis were done	Biofilm formation on glass surfaces and human dentin slices inhibited by 10, 30, or 50 µg/mL lipoteichoic acid Pre-formed biofilm disruption increased with lipoteichoic acid application in dose dependent manner Lipoteichoic acid potentiated antimicrobial action of endodontic medicaments CHX and CH	[109]

Table 1 (continued)

Probiotics	Methods	Results	References
Lact.plantarum ATCC 8014, *Lact. rhamnosus* ATCC 7469 and *Bifidobacterium bifidum* ATCC 11863	*Ent. faecalis* ATCC 29212 and *C. albicans* ATCC 10231 susceptibility was evaluated in planktonic and biofilm states by agar cup and CFU methods, respectively Deferred antagonism test was performed to identify probiotic products inhibitory to the pathogenic strains	Crude CFS had ZOI of 19.7 mm, 19.4 mm, and 18.2 mm with *L. plantarum*, *Lact. rhamnosus*, and *B. bifidum*, respectively, against *Ent. faecalis* ATCC 29212. Also, CFS diluted 1:2 had mean ZOI of 11.2 and 11.8 for *Lact. plantarum* and *Lact. rhamnosus*, respectively. No antimicrobial effect with 1:2 dilution CFS of *Bifidobacterium* group was evident following agar cup method Crude CFS of lactobacilli and *Bifidobacterium* had average ZOI of 19 and 18.3 mm, respectively, against *C. albicans* ATCC 10231. CFS diluted to 1:2, and CFS with pH adjusted to 6.0 displayed no antimicrobial effect against planktonic *C. albicans* *Lactobacillus* species reduced more than 90%, while *B. bifidum* reduced only 24.35% of *Ent. faecalis* biofilm *Lact. rhamnosus* was most effective (83.79%) in reducing *C. albicans* biofilm followed by *Lact. plantarum* (42.06%); *B. bifidum* had no anti-biofilm action against *C. albicans* biofilm Deferred antagonism activity was not observed in any group against both endodontic pathogens	[35]
Lactobacillus strain isolated from commercial probiotic cocktails (Ecobion and Darolac)	*Ent. faecalis* ATCC 29212 and *C. albicans* ATCC 10231 were used as endodontic pathogens Planktonic stage evaluation was done using the agar cup method and biofilm stage evaluation was performed by CFU evaluation following 72 h incubation of a mixture of the probiotic and pathogen on BHI agar	Both probiotic cocktails had significant antimicrobial action against both planktonic and biofilm form of *Ent. faecalis.* ZOI was 16.5 mm and 12 mm, and biofilm reduction were 90.6% and 77.5% for Ecobion and Darolac, respectively None of the probiotic cocktails had antimicrobial activity on *C. albicans* ATCC 10231, except Ecobion reduced *C. albicans* biofilms by 83.79%	[107]
Lact. plantarum and *Lact. rhamnosus*	*Ent. faecalis* ATCC 29212 was cultured with the probiotic strains in agar medium for 24 h, following which zones of growth inhibition were measured Deferred antagonism test was performed to evaluate release of bacteriocin-like inhibitory substances	Crude CFS of both *Lactobacillus* species had antimicrobial action with average zone of inhibition of 20 mm. 1:2 CFS dilution and CFS with pH adjusted to 6.0 had no antimicrobial action. Deferred antagonism test revealed no zone of inhibition	[108]

Another potentially beneficial mechanism associated with the use of probiotics involves quorum sensing disruption through gene regulation [121]. Quorum sensing is the central phenomenon by which microbes communicate in biofilms, and it regulates both bacterial colonization and virulence [122]. Reduced quorum sensing gene (vicKR and comCD) expression in *Strep. mutans* biofilms following exposure to probiotic strains of *Lactobacillus* species was reported by Wasfi and co-workers, and this is considered likely to play a role in the observed anti-caries effects of probiotics [123]. A schematic representation of antibiofilm activity of probiotics using different mechanisms has been demonstrated in Fig. 1.

Anti-inflammatory and Immuno-modulatory Activities

Inflammatory mediators released in response to microbial invasion of the root canal are important for the development and progression of the clinical manifestations of AP [124]. These mediators cause vasodilatation, increase vascular permeability, and recruit inflammatory cells. It has also been suggested that the increased inflammatory mediators seen in AP may also enhance systemic inflammation [125]. Inflammation-induced tissue damage may vary in different diseases; however, the markers that trigger this damage are very similar. They all act to increase the development of the inflammatory process, cause tissue destruction, and may even be directly involved in the onset of clinical symptoms [126].

The anti-inflammatory activity of individual probiotics is not always fully understood. Multiple factors can be involved, including strain type, duration of treatment, and dosage. The resultant anti-inflammatory actions are attributed to down-regulation and up-regulation of proinflammatory and anti-inflammatory cytokines, respectively, optimizing the balance between Th (T- helper) cells and Treg (T- regulatory) cells, and changes induced in antigen-presenting cells [127]. Moreover, it appears that some probiotics can also stimulate natural killer cells and dendritic cells to produce anti-inflammatory cytokines [128]. An effect on adaptive immunity, manifested by increased activation of lymphocytes and production of antibodies has also been reported [129]. The commensal and probiotic behavior of the oral probiotic *Strep. salivarius* K12 have been shown to be attributable to the bacterium (i) eliciting no proinflammatory response, (ii) stimulating an anti-inflammatory response, and (iii) modulating

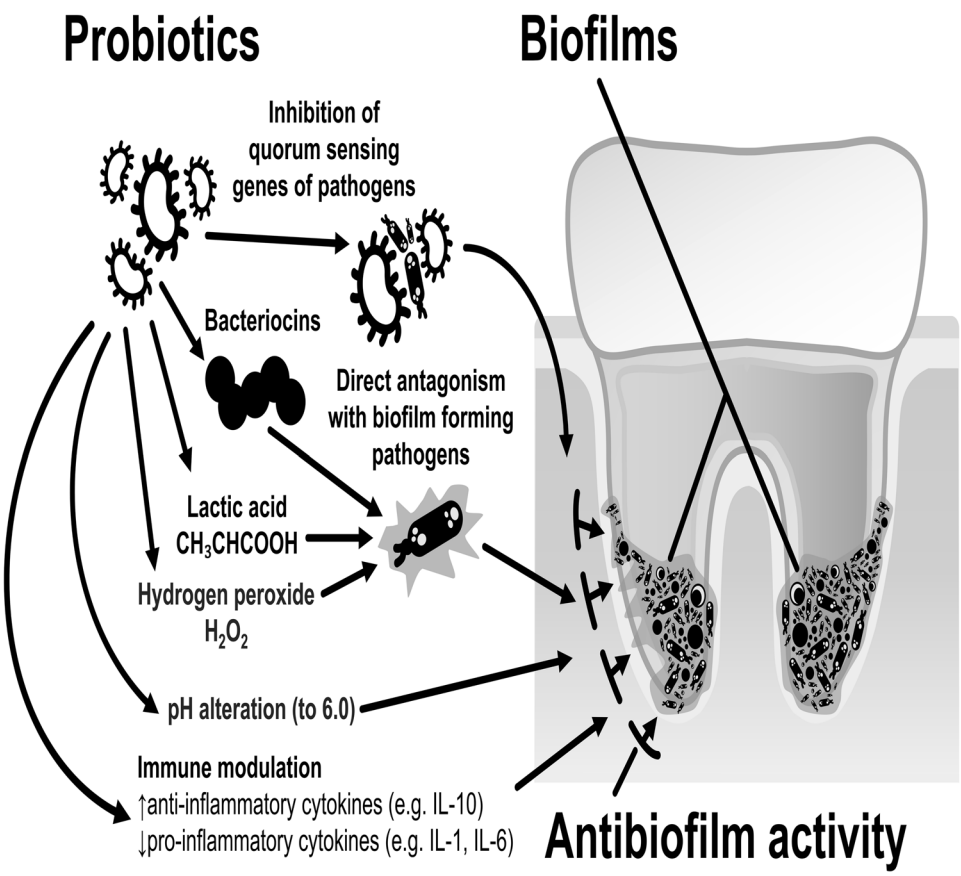

Fig. 1 Schematic representation of different mechanisms of probiotics action against biofilm formation. Biofilms formed by the pathogens may be inhibited due to reduced expression of quorum sensing genes of pathogens, production of antimicrobial compounds (such as bacteriocins, lactic acid, hydrogen peroxide), pH alteration, and immune modulation

genes associated with adhesion to the epithelial layer and homeostasis [130].

Autophagy is a natural process by which the body removes damaged cellular components and regulates metabolism. It also plays a role in immune activation [131] and acts to limit the immune response [132]. Impaired autophagy leads to the development of obesity, chronic inflammatory conditions of the gut, and periodontitis [133]. Some probiotics have shown promising results in the correction of faulty autophagy mechanisms in the gut, thereby helping to restore homeostasis [134]. The presence and participation of disturbed autophagy in periapical lesions [133] provide yet another target for probiotics in restoring periapical health. Yet another possible role for certain probiotics may relate to their ability to inhibit matrix metalloproteinase activity [135]. These molecules have a general involvement in tissue remodeling and wound healing and are responsible for the degradation of the extracellular matrix and base [21]. In periapical lesions they have been reported to participate in tissue destruction and lesion expansion [136].

Effect of Probiotics on Bone Resorption

It has been suggested that probiotics may affect bone metabolism via their anti-inflammatory effects and their facilitation of absorbance of nutrients and minerals in the gut [137]. Also, probiotics can increase the amount of OPG and decrease RANKL thereby decreasing bone resorption by RANKL-induced osteoclastogenesis [113]. Studies in rats have shown a beneficial role for probiotics in reducing alveolar bone loss [138], and in increasing mandibular bone density [139]. In another study, a positive effect on periodontitis-induced bone loss in mice was reported after topical treatment with *Lact. brevis* [140]. A significant reduction in mesial bone loss in rats was associated with administration of a strain of *Lact. reuteri* (both live and heat killed cells), underlining the potential beneficial role of probiotics in preventing bacterial-induced alveolar bone loss in the oral cavity [141].

As discussed earlier, Cosme-Silva et al. [113] reported a reduction in periapical bone loss associated with the use of oral probiotic supplements in mice. This was the first study to evaluate the role of probiotics in AP. The reason for the reduction in periapical bone loss was attributed to a favorable effect of probiotics on the OPG/RANKL system. In the presence of probiotics, a higher expression of OPG and lower production of RANKL was evident, thus contributing to reduced RANKL-dependent osteoclastogenesis. This report provides a useful initial lead on beneficial role of probiotics in preventing periapical bone resorption in AP. Follow-up studies in human subjects are now required to ascertain whether probiotics can help modulate the inflammatory and bone resorption processes associated with AP [70].

Prevention of Secondary Infection

Secondary infections in root canals are both prevalent and difficult to manage. Although these infections are more commonly caused by bacteria that have resisted primary treatment, dislodged or defective coronal seals may also lead to re-infection of the root canal space [106]. The limited effectiveness of root canal disinfectants is at least in part due to the complex morphology of the root canals and to the biofilm-associated nature of any bacteria colonizing the canals [42, 58]. Unfortunately, both the non-surgical and surgical retreatments that are currently performed for the management of persistent root canal infections are considered to be time-consuming, costly and have a relatively low chance of success [142]. A recent study projected the survival of teeth after non-surgical and surgical retreatment to be approximately 70% after a median follow up time of 10 years [143]. Therefore, prevention of secondary infection should be a focus of on-going patient care. Probiotics may have an adjunctive role to play in the prevention of secondary AP, based on the premise that they may help to inhibit colonization by pathogenic bacteria due to their antimicrobial and immunomodulatory properties [27]. Therapeutic applications of probiotics in the management of periodontitis have had promising outcomes [144]. Periodontal pathogens such as *F. nucleatum*, *P. intermedia*, *Ent. faecalis*, *Streptococcus* species, and *Parvmonas micra* are also found associated with secondary root canal infections, [36]. Thus, an implication of these studies is the potential beneficial role of probiotics in preventing secondary root canal infections.

Safety of Probiotics

Probiotics are widely considered to be safe for consumption by healthy adults. However, based on previous reports, the use of probiotics in immunocompromised patients entails a potential risk of sepsis and even shock [145]. Systemic infections are the major concern linked to probiotic use. Bacteremia arising after administration of *Lactobacillus* species such as *Lact. acidophilus*, *Lact. casei*, and *Lact.* GG [146] and fungemia associated with consumption of probiotic preparations containing *Saccharomyces boulardi* have been reported [147]. Reports of increased mortality in critically ill patients along with some minor gastrointestinal disturbances in the form of abdominal cramping, flatulence, and taste disturbances have also been reported [148]. Manipulation of the intestinal microbiome by probiotics may also impact adversely upon the digestion and absorption of nutrients. Furthermore, any long-term effects of probiotic usage on the immune system are currently unclear and

difficult to predict. Although the risks of probiotic usage by healthy adults appear to be minimal, the chances of transfer of latent antibiotic resistance determinants to pathogenic bacteria cannot be completely ruled out [121]. It is of course important to recognize that not all probiotic strains have a beneficial role in all health conditions. For example, *Lact. salivarius* W24, which is known to exhibit desirable antimicrobial and anti-inflammatory properties in the gastrointestinal tract, also has recognized cariogenic potential [146]. This helps to illustrate that the established benefits of one probiotic strain cannot be automatically extended to another probiotic candidate strain, even of the same species. Thorough selection and characterization of probiotic strains needs to be carried out specifically according to the targeted disease condition. Administration of probiotics in at risk populations such as immunosuppressed patients, pregnant females, patients with acute intestinal or bowel conditions, and patients with structural heart abnormalities should be closely monitored. In summary, there is clearly a need for more rigorous trial design involving careful safety assessment of probiotic strains [146].

Lacunae in the Literature

Ent. faecalis has been found to be a major endodontic pathogen in most reports of the potential antimicrobial role of probiotics. Studies have focused either on planktonic *Ent. faecalis* [107] or on both planktonic and biofilm stages of *Ent. faecalis* and *C. albicans* [35, 108]. Lipoteichoic acid purified from *Lact. plantarum* was evaluated either in multispecies biofilms [109] or in *Ent. faecalis* biofilms [110]. In both studies it was found to substantially increase the antimicrobial effect of commonly used medicaments such as CH and CHX. *Ent. faecalis* is the most commonly reported pathogen found associated with persistent root canal infections [43, 149], the reported prevalence being 8–71% by culture methodology and 10–90% by use of the more sensitive polymerase chain reaction (PCR) technique [150]. On the other hand, a recent study using next-generation sequencing (NGS) indicated that *Ent. faecalis* may not be as dominant a cause of primary or persistent root canal infections as had been previously considered [151]. Broader depth of coverage and detection of previously undetected bacteria are important advantages of NGS or high-throughput sequencing which allows for the accurate detection of species in clinical samples by independent and simultaneous sequencing of a large number of DNA fragments [152]. Based on findings of previous NGS studies, bacteria of the Proteobacteria [153] and Bacteroidetes [154] phyla are more frequently associated with persistent infection than are enterococci, which were found in fewer samples and in lower abundance [155]. However, a limitation of DNA-based molecular methods is that they detect the whole genome and are not able to discriminate between live and dead bacteria found in infected tissue. Biofilms detected in the root canal are usually multilayered, with a variable proportion of matrix and bacterial cells [42]. The microbial diversity in endodontic biofilms ranges from 10 to 20 species in primary infections to 1–5 species in persistent infections [15]. The detection of biofilms having greater microbial diversity, identification of a large number of previously undetected genera, and geographical variations in the root canal microbiota are key findings of recent studies in the field of endodontic microbiology. Only one of the studies identified in this review has used multispecies biofilms for assessing the antimicrobial efficacy of probiotics [109].

The composition of root canal biofilms is not static and the artificial substrates such as agar, glass plates and sliced dentin used in some studies do not adequately mimic the complexities of a root canal. Furthermore, the antimicrobial effects observed in agar diffusion tests do not necessarily translate into clinical reality [156]. Also, the failure to include standard endodontic disinfectants for comparison is another drawback of many reported studies. Lastly, the absence of human clinical studies highlights the information gap relating to the assessment of the utility of probiotics in AP. Therefore, caution should be exercised while extrapolating the current in vitro data to the diverse and vastly more biologically complex clinical conditions.

Future Directions

Future studies evaluating the application of probiotics in endodontics should focus on developing and applying more standardized protocols. Criteria for the selection of probiotics such as those suggested by de Melo Pereira et al. [70] may provide some guidance. They suggested that probiotics should tolerate local stress and adhere to the site of application. Furthermore, they should have anti-pathogenic activity and should be safe for human consumption, and lastly, clinical trials should support their application. Considering the development and application of probiotics is still in a nascent stage, the focus of future research should be on selecting and employing various clinically proven probiotic strains in exhaustive laboratory studies followed by animal and clinical trials. The localized delivery of probiotics inside the root canal using suitable vehicles providing stability, viability, and sustained release [157] is an exciting research and clinical prospect, and hopefully will prove successful in the management of AP.

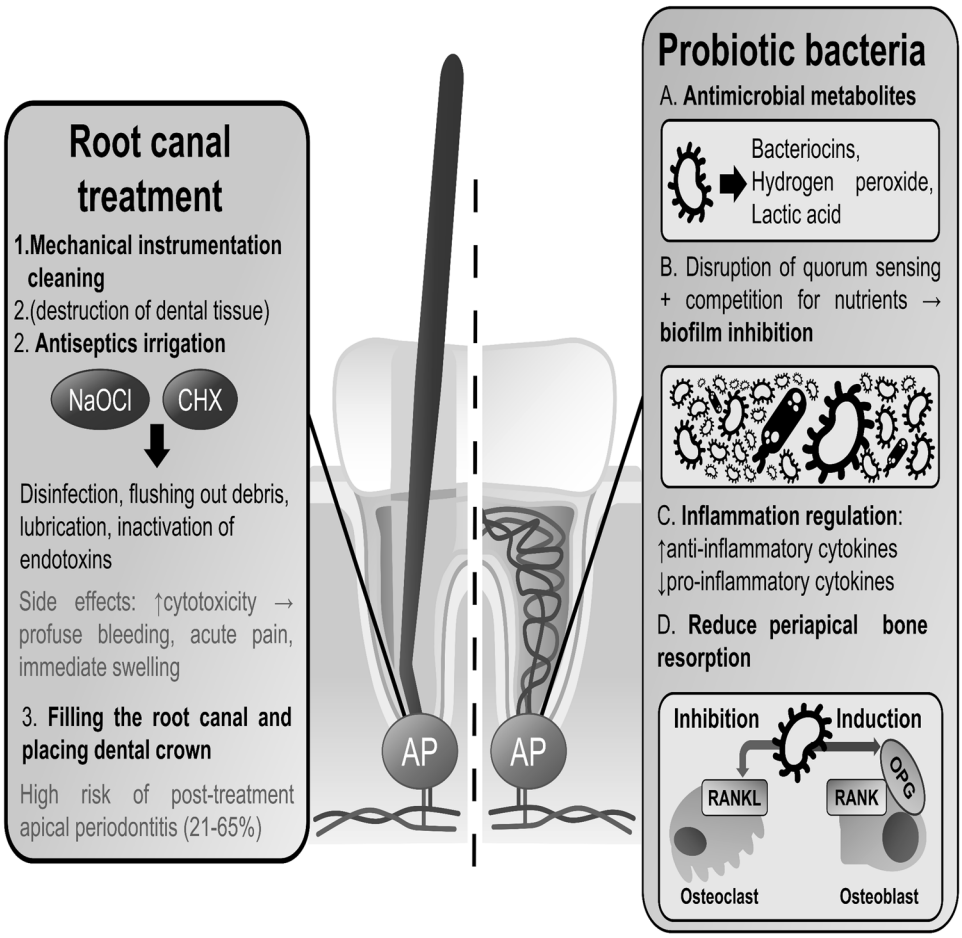

Fig. 2 Schematic representation of the current surgery and antiseptic-based treatment strategies for apical periodontitis and of the potential beneficial adjunctive role for probiotics in the management of these infections. The standard invasive protocol of mechanical instrumentation cleaning, intracanal antiseptics irrigation, and filling the root canal following placing of dental crown is compared with the putative roles of probiotics in immune modulation, as antimicrobials, inhibition of biofilm formation and reduction of bone resorption by promoting OPG expression and decreased RANKL–induced osteoclastogenesis. NaOCl sodium hypochlorite, CHX chlorhexidine, RANK receptor activator of nuclear factor kappa-B, RANKL RANK ligand, OPG osteoprotegerin

Conclusions

The existing literature documents some promising results supportive of a beneficial role for probiotics in countering the proliferation and pathogenic processes of endodontic pathogens within a biofilm matrix and in exerting, favorable anti-inflammatory effects and in reducing bone resorption (as shown in Fig. 2). However, it is imperative to stress that these studies have been characterized to date by considerable design heterogeneity in terms of the type, dose, duration and frequency of probiotic administration. Future research should now focus on developing standardized protocols and on the elimination of study design ambiguities potentially influencing the meaningful assessment of a role for probiotics in the management of AP.

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Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent For this type of study, formal consent is not required.

Conflict of Interest The authors declare that they have no conflict of interest.

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