

REVIEW

Probiotics: Evidence of Oral Health Implications

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The prevalence of common oral diseases, such as dental caries, periodontal diseases and oral candidiasis remains high in the general population. Various preventive strategies have been proposed and included in national health programs promoting oral health. Interest in probiotics in light of oral health has gradually evolved as attractive means in prevention of oral infectious diseases. The aim of the present review is to outline the current evidence on the role of probiotic species on oral health parameters and their beneficial role in contributing to healthier oral environment.

INTRODUCTION

Oral health is a vital component of health. World Health Organization defines it as a state of being free from chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft lip and palate, periodontal (gum) disease, tooth decay and tooth loss, and other diseases and disorders that affect the oral cavity.¹ Global health preventive programs adopted worldwide have substantially contributed to reducing the extent and the severity of common oral diseases. Yet the prevalence of periodontal diseases and caries, both having microbial components in their etiology, remain high. Caries affects practically everyone in the global population at some stages of life while 15 – 35% of the adult population in industrialized countries is estimated to have periodontitis.²

The role of host microbiota in individual's health has been developing as a concept for more than a century. In the early 1900s, Bond Stow emphasized the significance of *Lactobacillus bulgaricus*

on autointoxication and its importance on balancing gut health.³ The theory of Nobel laureate Ilya Metchnikoff (**Fig. 1**), that “we fight microbe with microbe”⁴) has attracted numerous followers who have presented clinical evidence on the role of lactic acid bacteria administration and their control on autointoxication-related mental conditions. However, this theory has been seriously discredited for several decades by famous supporters from the United States like Alvarez and Donaldson who ‘debunked’ autointoxication.⁵ The rapid scientific and technological advances in the new millennium have led to paradigm shift in understanding the relevance of entire body microbiome to human health and well-being. The role of beneficial healthy bacteria has been related to numerous pathological conditions of the gastrointestinal tract, skin, respiratory system and even mental health. Probiotics, which exert their health effect when digested in certain quantities, are able to promote health either directly or through their influence on other gut

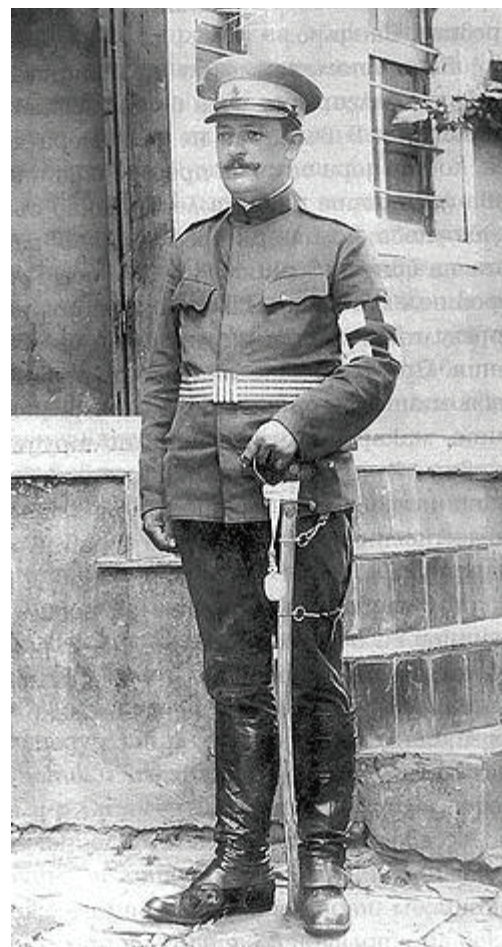
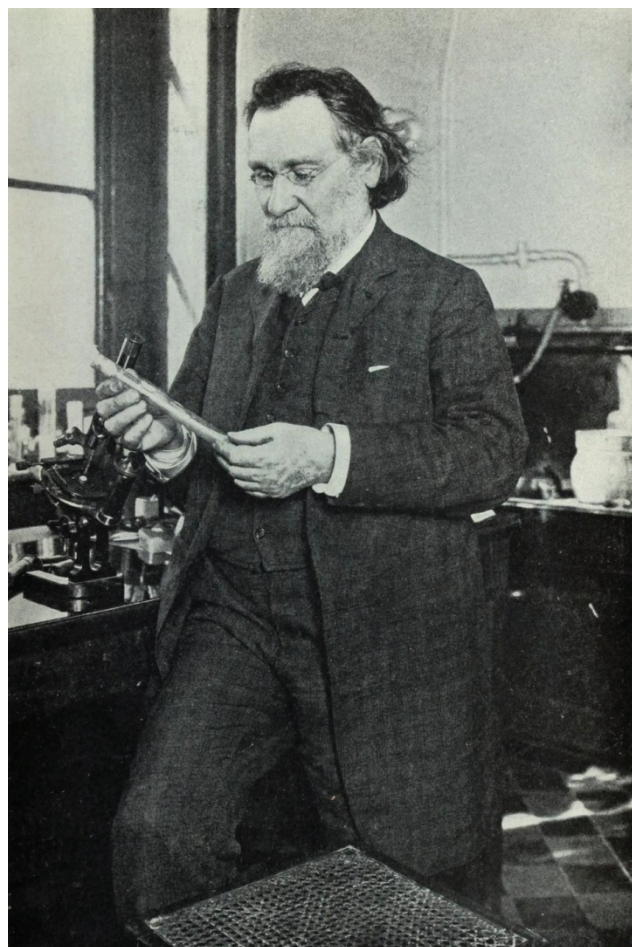


Figure 1. Ilya Metchnikoff, who laid the foundations of modern probiotics, and Stamen Grigorov, who isolated *Lactobacillus bulgaricus*, the main yoghurt starter.

microbes.⁶ The main mode of probiotic activity is ascribed to their influence on systemic cytokines, oxidative stress, and inflammatory markers upon oral consumption.⁷

Probiotics and their role on oral health has been proposed at the turn of the century and the reader is referred to two comprehensive review articles published in 2005 outlining the main scope and clinical relevance of healthy bacteria in the mouth.^{8,9} The aim of the present review was to outline and discuss the advances in probiotic research in relation to prevention and management of common oral diseases. **Table 1** lists probiotic species used in clinical trials in the field of dentistry.

PROBIOTICS AND DENTAL CARIES

Dental caries, although preventable, remains one of the most prevalent oral diseases in children and adults worldwide. Fernando et al.¹⁰ have suggested that dental caries in children could be a result of

possible interaction of risk factors such as maternal characteristics, environment, child's individual factors and epigenetics. Sugar and sugar-rich diet have been the main culprits for the development of carious lesions. A shift from the caries-promoting bacterium *Streptococcus mutans* only towards a more complex acid-tolerant consortium of oral bacteria leading to disease has been adopted lately. Molecular microbiological methods have shown that even with a sugar-rich diet a much broader spectrum of acidogenic microbes is found in dental biofilm.¹¹ Prevention of tooth decay by solely targeting *S. mutans* or by mainly limiting sugar intake is easily doomed to be inefficient in affecting disease progression. A more complex multidisciplinary approach including, for example, control of biofilm composition, control of dietary sugars in oral biofilm, and prolonged fluoride release, may be needed for caries control. Bacteriotherapy has emerged as such an alternative preventive measure which could target and tailor

Table 1. Probiotic species used in clinical trials to target oral diseases/conditions

Species	Condition	Number of subjects	Vehicle of administration	Reference
<i>L. rhamnosus</i>	Caries prevention	261	milk	Rodriguez et al. 2016
<i>L. reuteri</i>	Early carious lesions	36	tablet	Keller et al. 2014
<i>B. lactis</i>	Caries prevention	106	tablet	Taipale et al. 2012
<i>L. rhamnosus</i>	Root caries	160	milk	Petersson et al. 2011
<i>L. reuteri</i>	Chronic periodontitis	40	lozenge	Tekce et al. 2015
<i>L. reuteri</i>	Gingivitis	40	tablet	Iniesta et al. 2012
<i>L. salivarius</i>	Halitosis	20	tablet	Iwamoto et al. 2010
<i>L.salivarius</i>	Prevention of periodontitis	66	tablet	Shimauchi et al. 2008
<i>B. longum, S. thermophilus, L. bulgaricus</i>	Candida-associated stomatitis	65	mixture	Li et al. 2014
<i>L. rhamnosus</i>	Candida prevention	276	cheese	Hatakka et al. 2007
<i>L. brevis</i>	Halitosis	20	lozenge	Marchetti et al. 2015
<i>L. reuteri</i>	Halitosis	25	chewing gum	Keller et al. 2012
<i>L. reuteri</i>	Alteration to oral microbiota	44	lozenge	Romani Vestman et al. 2015

oral biofilm composition towards healthier microbial community. The use of *in vitro* and *in silico* biofilm models has allowed studies on bacteria in their similar-to-natural habitat, and respective changes in their habitat, to be investigated in more detail thus leading to more reliable outcomes.¹²

A primary requirement for a probiotic candidate is for that agent to establish itself in the respective environment. Our *in vitro* studies have shown that the probiotic *Lactobacillus rhamnosus* GG (LGG) could grow in dual- and multi-species oral biofilms affecting to various degrees the number of *S. sanguinis* and *S. mutans* in 4- and 2-species biofilm models, respectively.¹³ This integration of extrinsic probiotic species in oral community could be attributed to good adhesion properties and/or competition for binding sites with other species, as well as inhibitory activity against other members of the oral biofilm. Teanpaisan and coworkers have shown *in vitro* that caries pathogens could be effectively inhibited by oral lactobacilli with

Lactobacillus SD1-SD6 possessing the strongest inhibitory effect against *S. mutans* and *S. sobrinus*.¹⁴

LGG in different carriers, such as milk and cheese, has shown caries reducing effect in children.^{15,16} Caries preventive effect of LGG could be due to improved salivary buffering capacity and reduction of *S. mutans* counts.¹⁷ Salivary buffering capacity has also been observed to increase when healthy volunteers were given a probiotic tablet containing *L. salivarius*, thus allowing the authors to conclude that probiotic administration may lead to increased resistance to caries risk factors.¹⁸ Caries preventive effect of probiotics correlates with the time of their administration. Stensson et al.¹⁹ reported reduced caries prevalence and gingivitis score in primary dentition in 9-year-olds when the children had been given *L. reuteri* from birth and through their first year of life. *Bifidobacterium animalis* subsp. *lactis* BB-12 and *L. paracasei* F19, conversely, had no effect on caries occurrence if provided in infancy.^{20,21} Extrinsic probiotics rarely do establish

themselves in the mouth, but although transient, their presence may affect re-colonization of mutans streptococci after full-mouth disinfection as shown with *L. reuteri*.^{22,23} Except for their caries preventive potential, *L. reuteri* and *L. rhamnosus* have been tested to affect pre-existing carious lesions. No adverse effects were detected by administering *L. reuteri* but its intake insubstantially affected initial carious lesions.²⁴ However, a different outcome with reversal of primary root caries in healthy adults has been achieved with milk supplemented with *L. rhamnosus* and fluoride.²⁵

To conclude, the effect of probiotics on dental caries remains species-dependent and results differ between studies. More robust randomized clinical trials are needed before a universal probiotic strain, or possibly a combination of species, is found for the purposes of caries prevention and control.

PROBIOTICS AND PERIODONTAL DISEASES

Periodontal diseases including gingivitis and periodontitis originate in the presence of susceptible host challenged by the burden of pathogens in oral biofilm. They are clinically manifested by bleeding on probing, swelling, color alterations, pain, and in advanced stages tooth mobility is the leading symptom. Scaling and root planing and deep pocket debridement are primary treatment strategies which allow significant reduction of aggressive periopathogens. Although removing plaque by mechanical surface instrumentation is reliable and efficient treatment modality, the pocket re-colonization is highly unpredictable. In this regard, probiotics or beneficial microbes may find their place in tailoring treatment outcome after routine periodontal treatment. The periodontal pathogens in supra- and subgingival biofilm could be substantially reduced after probiotic lactobacilli administration as demonstrated by Mayanagi et al.²⁶

In subjects who have been clinically diagnosed with mild or moderate gingivitis probiotic administration is shown to decrease the volume of gingival crevicular fluid and bleeding on probing; clinical parameters such as gingival and plaque indices have also been positively affected.^{27,28} The observed changes are related to the reduced concentrations of inflammatory cytokines such as TNF- α and IL-8, caused by probiotics. The reduction in the number of periodontal pathogens after *L. reuteri* administration unequivocally contributed to improved parameters of gingival health.²⁹ Experimental gingivitis in healthy subjects, depending on the probiotic species tested, may show favorable outcome in terms

of lower amount of gingival crevicular fluid, less bleeding on probing, lower elastase and MMP-3 activities.^{30,31} However, daily intake of probiotic lozenges was not a winning strategy for experimental gingivitis when seven inflammatory mediators were measured.³²

The most commonly used probiotic species in chronic periodontitis patients so far is *L. reuteri*. It has been a useful adjunct to scaling and root planing when given as a lozenge once or twice daily. Lowering plaque index, gingival index, probing pocket depth and bleeding were the observed treatment outcomes.^{33,34} Again, the anticipated mechanisms of probiotic activity were associated with reduced numbers of periodontal pathogens, such as *Porphyromonas gingivalis*, and lower concentrations of TNF- α , IL-1 β and IL-17 in gingival crevicular fluid.^{35,36}

An emerging problem with the growing number of dental implants placed in general population is the occurrence of peri-implantitis and peri-implant mucositis which in their origin also are microbe-mediated. Attempt to use probiotics to combat such treatment complications has also been made. However, in these cases there is no clinical evidence to justify the use of probiotic supplements in addition to mechanical debridement and oral hygiene reinforcement.³⁷ As regards peri-implantitis, more studies with probiotic supplementation are indeed called for. In general, scientific evidence on probiotics vs. periodontal diseases still remains weak.

PROBIOTICS AND ORAL CANDIDA INFECTIONS

Another reasonably well explored field of probiotic applications is the chronic *Candida* infections of the soft tissues in the mouth. *Candida* species most commonly isolated from the oral cavity include *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. The growing antifungal resistance is becoming a serious problem worldwide and new strategies to combat pathogenic fungi in biofilm structures have been sought. *In vitro* studies have shown various inhibitory activity against oral *Candida* by different commercially available probiotic lactobacilli, with strongest inhibitory effect observed with LGG.³⁸ In an animal model study *S. salivarius* K12, a nonpathogenic commensal oral probiotic microorganism, significantly protected the mice from severe candidiasis by inhibiting the process of invasion of *C. albicans* into mucous surfaces or its adhesion to denture acrylic resins by mechanisms not associated with the antimicrobial

activity of the bacteriocin.³⁹ *Lactobacillus casei* and *Bifidobacterium breve* in yoghurt drink positively affect secretory IgA levels in saliva and significant reduction in *Candida* and non-*Candida* species has been observed in the oral cavity of elderly.⁴⁰ Frail elderly people living in nursing homes are among individuals highly susceptible to fungal infections. A *L. reuteri* lozenge given twice a day for 12 weeks led to a significant reduction of *Candida* counts in the oral cavities of elderly, although no notable effects on plaque index and bleeding on probing were defined.⁴¹ Similarly, probiotic combination of *Propionibacter freudenreichii* and LGG given in cheese reduced the risk for high salivary *Candida* counts by 75% compared with placebo cheese in a short-term intervention study on the elderly.⁴² However, the true value of probiotics in controlling oral *Candida* is still an open question.

PROBIOTICS AND HALITOSIS

Halitosis, or the unpleasant odour exhaled through the mouth, is a multifactorial condition which may have oral and/or non-oral origins.^{43,44} It is mainly attributed to biofilm accumulation on the dorsum of the tongue, the interdental spaces, and chronic inflammatory diseases of the periodontium. The prevalence of halitosis varies with estimated range between 22% to 50% in populations.⁴⁵⁻⁴⁹

Volatile sulphur compounds (VSC) like hydrogen sulphide and methyl mercaptans and other additional odours such as indole, skatole, putrescine and cadaverine are byproducts of food degradation by halitosis-producing microorganisms.⁵⁰ A rich source of VSC is the periodontal pocket densely populated by *Fusobacterium nucleatum*, *P. gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*, *Micromonas micros*, *Campylobacter rectus*, and *Eikenella corrodens*.⁵¹⁻⁵³ The intensity of clinical bad breath has been correlated directly to periodontal health status.⁵⁴ Nevertheless, true evidence of such relationship has been questionable^{54,55}, and tongue microbiota may play a greater role in terms of oral malodor in comparison to subgingival bacteria.⁵⁶⁻⁵⁸ Halitosis could also be associated with dietary habits and consumption of foods like garlic, onions or spiced foods is a well understood reason for this. Certain diseases, such as diabetes and uremia, and medications could also contribute to bad breath.⁵⁹⁻⁶¹

Halitosis affects to various degrees key aspects of person's quality of life reflecting in social relationships and emotional well-being. Treatment of

halitosis is achievable once its etiology is properly diagnosed. Self-care products such as chewing gums, mouthwashes, mouth sprays and toothpastes can mask temporarily the unpleasant odor and improve the satisfaction of the individual. However, the emphasis remains on professional treatment and strategies at reduction and/or elimination of VSC producing microorganisms.

Burton et al.⁶² were the first to address the potential of oral probiotics in reduction of halitosis. By administering *S. salivarius K12* in lozenges after initial chlorhexidine rinse, eight out of 13 healthy individuals maintained reduced breath levels of VSCs for at least 2 weeks. Pre-emptive colonization of the oral cavity with competitive commensal bacteria following mechanical and chemical pretreatment has been the aim of Burton et al.⁶³ when combating halitosis with *S. salivarius K12*. Additionally, salivaricin production by probiotic species could cross-stimulate salivaricin production by other pre-existing members of the host microbiota, thus establishing lower levels of VSC. Inhibitory activity of *S. salivarius K12* on Gram-positive species involved in halitosis is another mode of activity to be utilized when targeting bad breath.⁶⁴ A cross-over trial including 23 participants has shown *L. salivarius WB21* to have a positive effect on oral malodor parameters by using organoleptic test score.⁶⁵ Moreover, the same species has shown beneficial effects on bleeding on probing from the periodontal pocket. Administering probiotics with chewing gums could also reduce bacteria that produce malodorous compounds other than VSCs as demonstrated by Keller et al.⁶⁶ After 14 days of treatment, the organoleptic scores were significantly lower in the *L. reuteri* group compared with placebo. *In vitro* testing of yogurt starter *S. thermophilus* could reduce generation of VSCs by *P. gingivalis* possibly by suppressing its growth, and the whole bacteria and the spent culture medium of *S. thermophilus* reduced emission of VSCs.⁶⁷ A well-studied probiotic species *L. brevis CD2*, however, showed no effect on halitosis after 14-day treatment in a randomized, double-blind placebo-controlled clinical trial.⁶⁸ However, although potentially promising, the results from the above mentioned studies there still is no sound evidence to support recommendation of probiotics for the treatment of halitosis.

CONCLUSION

Evidence of beneficial effects of probiotics on

oral health is accumulating. In general, the growing population awareness and demand for healthy living inevitably leads to broader availability of health-promoting foods on the market. In this field probiotics have become fashionable among the public and are marketed as one of the key elements in well-balanced general health. As here discussed, most oral health effects of probiotics have been observed after administration of strains which are not natural members of oral microbiota. Possibly a more attractive and reliable probiotic might be found among species which are natural members in the host microbiome. Screening of such probiotic candidates in human oral bacterial populations is ongoing with some evidence already available.⁶⁹ Further, multi-center clinical trials with enough statistical power, and the use of high-throughput analytical tools, are needed for true evidence before the role of probiotics on oral health can be established and their use justified. Currently the evidence yet remains weak.

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Пробиотики: Доказательства о последствиях для стоматологического здоровья

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Распространение таких наиболее часто встречающихся стоматологических заболеваний как зубной кариес, пародонтит и оральный кандидоз остаётся по-прежнему высоким среди населения. Большое количество разных стратегий для профилактики данных заболеваний были предложены и включены в национальные программы здравоохранения, нацеленные на стоматологическое здоровье. Интерес к пробиотикам в свете стоматологического здоровья постепенно возрос в качестве привлекательного средства для профилактики стоматологических инфекционных заболеваний. Целью настоящего обзора является рассмотрение современных доказательств о роли пробиотических штаммов в отношении параметров стоматологического здоровья и их полезной роли в отношении более здоровой среды полости рта