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Artigo de Revisão Bibliográfica

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"Probióticos em Medicina Dentária e Oral: tendências recentes"

"Probiotics in dentistry and oral medicine: recent trends"

Cláudia Sofia da Silva Campos

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“Probióticos em Medicina dentária e oral: tendências recentes”

“Probiotics in dentistry and oral medicine: recent trends”

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Artigo de Revisão Bibliográfica

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Once more, with feeling

"Even if you see them coming, you're not ready for the big moments. No one asks for their life to change, not really. But it does. So what – are we helpless puppets? No. The big moments are 'gonna come. You can't help that. It's what you do afterwards that counts. That's when you find out who you are"

Buffy the Vampire Slayer

Acknowledgments

First, I'd like to thank my beloved parents for their unwavering support. I wouldn't be able to accomplish anything without them.

Then, I would also like to thank both by advisor and co-advisor for their supervision and constructive criticism.

Resumo

Probióticos são microrganismos benéficos que auxiliam na modulação de agentes patogénicos, contribuindo para a prevenção ou tratamento de doenças. Acredita-se que podem ser capazes de substituir tratamentos associados a um maior número de efeitos adversos, ou ser complemento de outros, melhorando a sua eficiência. Por isso o objetivo deste trabalho é a pesquisa e compilação de provas concretas da eficiência e aplicabilidade de probióticos no tratamento de doenças no âmbito da medicina dentária.

Para isso foram avaliados 61 ensaios clínicos produzidos na última década (2009-2019), incidindo na intervenção sobre cárie dentária, doença periodontal, infeções fúngicas por *Candida albicans*, líquen plano e mucosite.

A prevenção da doença (cárie dentária e doença periodontal) foi o objetivo em 52,4% dos estudos (n=33), enquanto que o tratamento de cárie dentária e periodontite ativas foi o foco na restante amostra. Os estudos relacionados com C*andida albicans* focaram-se essencialmente em populações idosas, que já tinham maiores taxas de colonização pelo fungo, e os seus objetivos eram a redução da carga microbiana e da sintomatologia associada (n=6). Por outro lado, a maioria dos ensaios clínicos focados no tratamento e prevenção de cáries recorreram a populações jovens (crianças em idade escolar) e os focados na periodontite, recorreram a adultos. As estirpes probióticas mais utilizadas foram *Lactobacillus reuteri* (27%, n=17), *Lactobacillus rhamnosus* (11,1%, n=7), *Lactobacillus casei* (9,5%, n=6), *Lactobacillus paracasei* (6,3%, n=4). Globalmente, em 28 ensaios, as estirpes escolhidas foram capazes de melhorar um sintoma associado a uma das doenças supracitadas, em 30 ensaios foram capazes de modular o microbioma oral e em 8 ensaios provou-se terem sido capazes de estimular o sistema imunitário do hospedeiro. Em geral, a ação probiótica foi apenas parcialmente bem-sucedida, pois não foi efetiva em todos os parâmetros que os ensaios se propuseram melhorar, o que indica que a utilização de probióticos poderá ser mais eficiente quando administrada em conjunto com outros tratamentos e protocolos já utilizados, especialmente no que toca à prevenção e tratamento de cárie dentária em crianças e como coadjuvantes no tratamento das causas e sintomas da doença periodontal.

Palavras chave

Probióticos, *Lactobacillus reuteri, Lactobacillus rhamnosus, Llactobacillus casei,* medicina dentária, medicina oral, cárie dentária, doença periodontal, *Candida albicans*, mucosite, líquen plano

Abstract

Probiotics are beneficial microbes that can help to modulate the proliferation of pathogens and prevent or treat disease. Probiotics are believed to be able to substitute treatments with a heavy load of side effects or aid others, improving their effectiveness. Hence, this study's objective is the research and complication of concrete evidence proving that probiotics can effectively be applied in dentistry and oral medicine.

In order to do so 61 clinical trials performed during the last decade (2009-2019) were evaluated regarding caries, periodontal disease, *Candida albicans* infections, lichen planus and mucositis were assessed in this matter.

Disease prevention (caries and periodontitis) was the objective in 52,4% (n=33) of trials, while the treatment of active caries and chronic periodontitis was the goal in the remaining sample. The studies regarding *C. albicans* usually relied on an older population, which already had higher counts of the fungi, and their objective was reducing symptoms and microbial load (n=6). On the other hand, most caries trials were based on school aged children and periodontitis in adults. The most used probiotic strains were *Lactobacillus* reuteri (27%, n=17), *Lactobacillus rhamnosus* (11,1%, n=7), *Lactobacillus casei* (9,5%, n=6) and *Lactobacillus paracasei* and *Lactobacillus crispatus* (both with 6,3%, n=4). Globally, in 28 trials, the probiotic strain was successful in improving a clinical symptom, in 30 they were able to modulate the surrounding microbiome and in 8 they were able to stimulate the host's immune response. Probiotics were often only partially successful, indicating that their most effective administration is in conjunction with already established protocols, especially when it comes to caries disease progression in children as well as in supporting the treatment of causes and symptoms of periodontal disease.

Key Words

Probiotics, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus casei,, dentistry, oral medicine, caries, periodontal disease, Candida albicans, mucositis, lichen planus

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Abbreviations list

L. – *Lactobacillus*

C. albicans – *Candida albicans*

Spp – species

BOP – bleeding on probing

PD – Probing depth

GI – Gingival Index

PI – Plaque index

SRP – Scaling and root planning

Salivary IgA - Salivary immunoglobulin A

CFU - colony forming unit

1. Introduction

1.1. Probiotics – an overview

According to the World Health Organization (WHO), probiotics are living microorganisms that "when administered in adequate amounts, confer a health benefit on the host". These microorganisms are generally lactic acid bacteria (LAB), meaning that metabolize sugars into lactic acid trough fermentation. Probiotic LAB mainly belongs to the Firmicutes (*Lactobacillus, Lactococcus, Staphylococcus, Streptococcus*) and Actinobacteria phylum (*Bifidobacteria*). This study aims to summarize the most recent clinical trials applying probiotics to oral health and possibly offer a therapeutic alternative or addition to already existing treatments.

Lactobacillus are gram positive, non-spore forming, catalase negative bacteria. They generally have low cytosine plus guanine (CG) content and are facultative anaerobes. Taking fermentation processes as a taxonomic criterion, the *Lactobacillus* group can be divided in the homofermentative, facultative heterofermentative and heterofermentative groups. The organisms in the homofermentative group exclusively transform hexoses into lactic acid trough glycolysis. On the other hand, heterofermentative bacteria can use a wider variety of sugars (pentoses) to produce other byproducts (CO_2, acetic acid, ethanol), using O_2 as a growth stimulator and electron acceptor, which results in greater ATP formation (Charalampopoulos and Rastall 2009, Lahtinen, Salminen et al. 2012). In table 1 the main probiotic strains used in oral health are presented:

Table 1: Fermentation processes of the main oral probiotics (*lactobacillus*)

Homofermentative	Facultative heterofermentative	Heterofermentative
	Lactobacillus casei	
Lactobacillus acidophilus	*Lactobacillus paracasei*	*Lactobacillus brevis*
Lactobacillus Jonhsonii	*Lactobacillus rhamnosus*	*Lactobacillus fermentum*
Lactobacillus crispatus	*Lactobacillus curvatus*	*Lactobacillus reuteri*
Lactobacillus gasseri	*Lactobacillus plantarum*	
	Lactobacillus salivarius	

Adapted from S. Lahtinen, A.C. Owehand et al "Lactic Acid Bacteria. Microbiological and functional aspects"

The probiotic's influence on extracellular pH is their major form of action. Lactic acid production has an inhibitory effect on many pathogenic organisms by causing the dissociation of small fatty acids. These penetrate the cellular membrane and disrupt microbial metabolism. The acids produced by heterofermentative lactobacilli aren't as strong (Charalampopoulos and Rastall 2009, Lahtinen, Salminen et al. 2012).

Bifidobacterium differ from *lactobacilli* because they use a specific enzyme (fructose-6-phosphoketolase) to degrade hexoses into lactic acid. They are also heterofermentative, non-spore forming anaerobes. They have strong adhesion capabilities and are safe for consumption (Charalampopoulos and Rastall 2009, Lahtinen, Salminen et al. 2012).

Lactobacillus fermentum, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactobacillus plantarum* can be normally found in human saliva or dental plaque, even though only accounting for 1% of cultivable microbes. It is believed that their positive effects, when administered in higher numbers that usual, are pH reduction, inhibition of pathogens in dental biofilm, antimicrobial substance production, nutrient and adhesion sites competition with oral pathogens, immunomodulation of the host's response and improvement in mucosal permeability. The reduction in oral pathogens can be achieved both by pH decrease and the probiotic's production of antimicrobial products – bacteriocins; for example, reuterin 6, produced by *Lactobacillus reuteri* (Charalampopoulos and Rastall 2009, Lahtinen, Salminen et al. 2012).

In addition, probiotics can improve immunity functions by adhering to epithelial cells in the mucosa. Cell structures such as fimbriae and surface proteins bind to mucine, glycoproteins and human fibronectin. *L. acidophilus* has "Mub proteins" that adhere to fibronectine, while *L. rhamnosus* has "Spac pilin" (pili) that connects with mucus and aids its persistence in the gastrointestinal tract when ingested. This adds to acid and bile resistance of *L. rhamnosus*. Some oral benefits can be attained with probiotic's presence in the gut, but their persistence in the oral cavity is an objective whenever local lesions are to be treated – such as caries. Hydrophobic nature probiotics have better adhesion properties and can connect with salivary mucin. *Lactobacillus paracasei* are the most hydrophobic potentially beneficial microbes isolated from tooth surfaces. On the gingiva, *lactobacilli* congregate in the presence of ammonia and can either positively regulate

plaque formation, ore enter a symbiotic relationship with pathogens and cause disease (Banerjee, Sengupta et al. 2016). There are also more systemic effects in the administration of oral probiotics. The immunostimulation in healthy patients can be measured as increased cellular activity and increase in serum and mucosal antibodies - mostly IgA, but also IgM and IgG - and cytokines collected both from salivary and crevicular gingival fluid samples (Greenberg, Glick et al. 2008).

Regarding oral health, probiotics have 3 major applications: the prevention and treatment of caries, periodontal disease and *Candida albicans* infections. Inflammatory and immune diseases such as some types of mucositis and lichen planus are also sometimes addressed.

1.2. Caries

Caries lesions arise from a group of different variables: the host (dental morphology and mineral composition; salivary composition), the oral microbiome (cariogenic pathogens, plaque and plaque pH) and the environment (frequency of ingestion and types of carbohydrates). *Streptococcus mutans* and *Streptococcus sobrinus* are the most frequently isolated species in cavity lesions, especially in the pre cavity phase. *S. mutans* can only trigger disease in high quantities, as it is indigenously present in many regions of the oral cavity. They have the capability to adhere to non-flaky surfaces, such as teeth, and synthetize extracellular polysaccharides and begin the process of plaque formation. At the same time, they metabolize sucrose and produce acid (mostly lactic acid) that demineralizes teeth surfaces and lowers salivary pH, producing cavitation (Melo 2001). *L. salivarius, L. plantarum, L. paracasei, L. rhamnosus,* and *L. fermentum* were shown to have antimicrobial activity against *S. mutans* (Koll, Mandar et al. 2008).

1.3. Periodontal disease

Periodontal disease is caused by microorganisms and leads to inflammation, destructing dental support tissues: bone, periodontal ligament and gingiva. Infragingival plaque is pathological because it can't be easily removed at home, it promotes tissue invasion and is a source of endotoxins and exotoxins produced mostly by: *Agregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Prevotella intermedia* and *Tannerela forsythia* (Lindhe, Lang et al. 2008). A more comprehensive overview can be seen in table 2.

Table 2: Virulence factors of the most common periodontal pathogens

Pathogen	Virulence factors	Detection sites
Aggregatibacter actinomycetemcomitans	Leukotoxin, catalase and superoxide dismutase production Endotoxins Invasion of epithelial and endothelial cells	Detected in high counts in some chronic periodontitis lesions
Porphyromonas gingivalis	Superoxide dismutase production LPS and adhesins Proteolytic enzymes that destroy connective tissue Fimbriae Invasive capabilities: alkaline phosphatase (bone invasion) Bacteriocins	Highly related with periodontal disease – not present in regular oral microbiota
Tannerella forsythia	Invasive capabilities Shares antigens with *P. gingivalis*	Detected in high counts in some refractory chronic periodontitis, as well as in abscesses and active lesions
Prevotella intermedia	LPS and adhesins Proteolytic enzymes Fimbriae	Detected in high counts in ulcerative gingivitis and refractory periodontitis
Treponema denticola	Endotoxins and proteolytic enzymes Mobility Diminishes lymphocyte response	The main pathogen of ulcerative gingivitis and active periodontitis lesions
Fusobacterium nucleatum	Endotoxins and leukotoxins Inhibits leucocyte quimiotaxis	Detected in high counts in chronic periodontitis and abscesses

Adapted from J. Lindhe, N.P Lang et all "Clinical Periodontology and Implant Dentistry

There is some data that implies that probiotic organisms have the capability to disrupt plaque formation, by interfering with its pathogens. As it has been referred, LAB produce many antimicrobial substances; for example, *L. reuteri* produces hydrogen peroxide (Szkaradkiewicz, Stopa et al. 2014, Tobita, Watanabe et al. 2018). Furthermore, *L. rhamnosus* have a strong inhibitory effect against cariogenic species and gram-negative periodontal pathogens (Morales, Carvajal et al. 2017). And *L. brevis* has the capability to prevent nitric oxide production, and hence inhibit gingival inflammation (Lee, Kim et al. 2015). *Streptoccocus* spp. is able to proliferate in periodontal pockets after root scaling, avoiding the recolonization of such sites by unwanted species (Laleman, Yilmaz et al. 2015).

Other than the epithelial barrier itself, the organism has innate defenses – saliva and the inflammatory process, and specific responses – cellular and humoral immunity. For example *L. plantarum* L-137 is capable of inducing IL-12, which leads to a Th1

immune response and the production of type I IFN in humans (Iwasaki, Maeda et al. 2016). And *Bifidobacterium animalis* decreased the levels of IL-1 β in gingival crevicular fluid (GCF) in simulated plaque formation after a 5-day no brush period (Kuru, Laleman et al. 2017).

1.4. Mucositis

Oral mucositis is an inflammatory condition on the mucosa. Its pathogenesis is mainly correlated with an external aggression and an increase in cytokine production that affects connective tissue. There is increased growth of *S. mutans, lactobacilli, C. albicans* and gram-negative bacilli, that may result in oral infections. Some probiotic strains are expected to be able to control these microbial populations by direct competition or the production of bacteriocins (Neville, Damn et al. , Greenberg, Glick et al. 2008).

1.5. *Candida albicans* infection

The pathological proliferation of *C. albicans* is called candidiasis, and it is the most common form of fungal oral infection in humans. Prosthetic stomatitis tends to be grouped with erythematous candidiasis because both have a characteristic mucosal erythema. Nevertheless, prosthetic stomatitis is mostly related with older patients and some level of neglect in their denture's hygiene, while the erythematous type is more correlated with systemic conditions, such as cancer treatment (Neville, Damn et al.).

The environment provided by the combination of oral mucosa and denture surface is ideal for the growth of this species: nutrient rich, with a decreased flow of oxygen and saliva and with a nonrenewable (acrylic) surface on which the fungus can attach itself and proliferate. *C. albicans* is associated with the development of denture stomatitis but other pathogens such as *S. mutans* can aid its adhesion to the tissue/dentures. *S. mutans* produces an extra cellular matrix polysaccharide that facilitates the attachment of other microorganisms. Mucosal infection begins when the fungus adheres to epithelial cells – for example, when an ill-fitting denture causes friction and disrupts the epithelium – or due to systemic diseases such as poorly controlled diabetes.

The infection may also arise due to the immunocompromised state of the host, triggered by radiotherapy and chemotherapy. Patients receiving cytotoxic drugs are highly susceptible to fungal infections, that not only cause pain and discomfort, but can also extent to the esophagus leading to disseminated candidiasis (Lashof, Bock et al. 2004). As for radiation therapy, the decrease in saliva production is a well-known

predisposing factor for candidiasis. Radiotherapy to a dose of 50-60 Gy generally tends to cause lifelong damage to the salivary glands, and hence, permanent xerostomia (Rautemaa, Rusanen et al. 2006).

1.6. Lichen Planus

Lichen planus is a mucocutaneous disease with immunological mediation: auto reactive T cells that cannot distinguish between host cells and foreign antigens are activated triggering the agents of the inflammatory process (Neville, Damn et al. , Greenberg, Glick et al. 2008). It's erosive form is usually treated with corticosteroids that can lead to C. *albicans* infection (Neville, Damn et al.). And, as recent study discusses, probiotics are able to diminish microbial infection and suppress T cell activation and proliferation, as well as diminishing keratinocyte apoptosis and modulating the production of inflammatory cytokines, MMP-9 expression and mast cell degranulation (Han, Zhang et al. 2017).

2. Materials and methods

This study aimed to examine recent clinical trials regarding probiotics and oral health care. The search was performed on PubMed's database, with the following criteria: Clinical trials published between 2009 and 2019, in human subjects. Table 3 shows the results of the search, by target disease:

Table 3: Search terms

Target disease	keywords	Number of trials
Caries	"Probiotics" AND "caries	n=28
Periodontal disease	"Probiotics" AND "periodontal disease[1]" OR "Periodontitis"	n=26
Yeast infections[2]	"Probiotics" and "oral yeasts"	n=1
	"Probiotics" and "Candida"	n=20
Mucositis	"Probiotics" and "Mucositis"	n=6
	"Probiotics" and "Mucosistis" and "Neoplasms"	
Lichen planus	"Probiotics" and "lichen planus"	n=1

[1] Periodontitis as a broader term that includes gingivitis

[2] The use of the term "fungi" yielded no results regarding exclusively the oral cavity

As for exclusion criteria, trials that evaluated the performance of probiotics or the treatment of diseases outside the oral cavity weren't addressed. In the case of mucositis, most trials regarded mucositis in the context of implantology, and not as result of other etiologies – cancer treatment, for example. This meant that most studies (n=5) in this category were also found in the context of periodontology and probiotics. The same for lichen planus, whose only trial also discussed *C. albicans* infection. Then the search for *C. albicans* infections and probiotics yielded 20 results of which 6 concerned the oral cavity. In the end, 61 trials met the criteria to be included in this study.

Descriptive statistical evaluation was performed in order to convey the major trends seen in probiotics applied to oral health in the last decade. So, the trials were summarized in a series of variables: intervention period, sample size, probiotic strain used, form of probiotic administration, target disease and the existence of positive statistically significant outcomes in terms of microbiological modulation, improvement of clinical signs and/or the host's immune response.

Study variables varied across trials. Clinical variables for caries were cavitated lesions, remineralization of white spots and plaque index (PI). Some studies also addressed gingival health, though it wasn't the focus. Microbiological variables were evaluated by assessing the reduction of cariogenic microorganisms. Whenever the long-term permanence of a *Lactobacillus* strain was assessed it referred to the probiotic strain itself and not the possible pathogen. As for periodontal diseases (chronic periodontitis, gingivitis and peri-implant mucositis), clinical success was evaluated mainly as a reduction in probing depth (PD), bleeding on probing (BoP), clinical attachment loss (CAL), gingival index (GI) and plaque index (PI). Then the effects on the microbiome were based on the reduction of periodontal pathogens. Immunological variables were also addressed in some clinical trials regarding periodontitis, mostly the presence of inflammatory cytokines in GCF and saliva. Further explanation in table 4.

Table 4: Tested variables

Target disease	Clinical variables	Microbiological variables	Imunological variables
Caries	**Caries increment** (Stecksen-Blicks, Sjostrom et al. 2009, Stensson, Koch et al. 2013, Hedayati-Hajikand, Lundberg et al. 2015, Wattanarat, Makeudom et al. 2015, Rodriguez, Ruiz et al. 2016, Villavicencio, Villegas et al. 2017) **White spot leseions (WSL)** (Gizani, Petsi et al. 2015) **Early caries lesions (changes in enamel fluorescense)** (Keller, Nohr Larsen et al. 2014) **Salivary flow** (Nishihara, Suzuki et al. 2014) **Primary root caries lesions (PRCL)** (Petersson, Magnusson et al. 2011)	**Salivary *S. mutans* and *Lactobacillus* counts** (Chuang, Huang et al. 2010, Aminabadi, Erfanparast et al. 2011, Jindal, Pandey et al. 2011, Singh, Damle et al. 2011, Cildir, Sandalli et al. 2012, Glavina, Gorseta et al. 2012, Mortazavi and Akhlaghi 2012, Stensson, Koch et al. 2013, Gizani, Petsi et al. 2015, Villavicencio, Villegas et al. 2017, Alamoudi, Almabadi et al. 2018, Tobita, Watanabe et al. 2018) **Salivary *S. mutans* counts** (Juneja and Kakade 2012, Romani Vestman, Hasslof et al. 2013, Taipale, Pienihakkinen et al. 2013, Teanpaisan and Piwat 2013, Ghasemi, Mazaheri et al. 2017, Pahumunto, Piwat et al. 2018)	**Salivary buffer capacity** (Chuang, Huang et al. 2010, Glavina, Gorseta et al. 2012, Nishihara, Suzuki et al. 2014, Villavicencio, Villegas et al. 2017) **Salivary IgA** (Stensson, Koch et al. 2013) **Salivary HNP1-3 levels** (Wattanarat, Makeudom et al. 2015)
Periodontal ilnessess	**Gingival index (GI) and Bleeding on probing (BOP)** **Plaque index (PI)** **Probing depth (PD)** **Clinical Attachment loss (CAL)** (Shimauchi, Mayanagi et al. 2008, Mayanagi, Kimura et al. 2009, Harini and Anegundi 2010, Iwamoto, Suzuki et al. 2010, Teughels, Durukan et al. 2013, Szkaradkiewicz, Stopa et al. 2014, Toiviainen, Jalasvuori et al. 2014, Flichy-Fernandez, Ata-Ali et al. 2015, Hallstrom, Lindgren et al. 2015, Kraft-Bodi, Jorgensen et al. 2015, Laleman, Yilmaz et al. 2015, Lee, Kim et al. 2015, Alkaya, Laleman et	***Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum* gingival counts** (Mayanagi, Kimura et al. 2009, Teughels, Durukan et al. 2013, Ince, Gursoy et al. 2015, Alkaya, Laleman et al. 2016, Alanzi, Honkala et al. 2017, Galofre, Palao et al. 2017, Montero, Iniesta et al. 2017, Morales, Gandolfo et al. 2017, Sajedinejad, Paknejad et al. 2017, Tobita, Watanabe et al. 2018, Tartaglia, Tadakamadla et al. 2019)	**Peri implant crevicular fluid** (Flichy-Fernandez, Ata-Ali et al. 2015) **Peri implant concentrations of inflamatory citokines** (Flichy-Fernandez, Ata-Ali et al. 2015) **GCF cytokines** (Szkaradkiewicz, Stopa et al. 2014, Hallstrom, Lindgren et

	al. 2016, Iwasaki, Maeda et al. 2016, Mongardini, Pilloni et al. 2016, Schlagenhauf, Jakob et al. 2016, Alanzi, Honkala et al. 2017, Galofre, Palao et al. 2017, Kuru, Laleman et al. 2017, Montero, Iniesta et al. 2017, Morales, Carvajal et al. 2017, Sajedinejad, Paknejad et al. 2017, Tada, Masaki et al. 2017, Tobita, Watanabe et al. 2018) **Halitosis** (Iwamoto, Suzuki et al. 2010) **GCF volume** (Kraft-Bodi, Jorgensen et al. 2015, Kuru, Laleman et al. 2017) **Papilla bleeding Index and Interproximal plaque index** (Staab, Eick et al. 2009)	**Salivary *S. mutans* and *Lactobacillus* counts** (Toiviainen, Jalasvuori et al. 2014)	al. 2015, Keller, Brandsborg et al. 2017, Kuru, Laleman et al. 2017) **TNF-a blood counts** (Schlagenhauf, Jakob et al. 2016) **Salivary Lactoferrin** (Shimauchi, Mayanagi et al. 2008) **GCF elastase, MPO and MMP-3 activity** (Staab, Eick et al. 2009)
Fungal Infections	**Mucosal symptoms** **VAS-pain** **OLP severity score** **Plaque index (PI)** **Gingival index (GI)** (Kraft-Bodi, Jorgensen et al. 2015, Keller and Kragelund 2018) **Tongue and mucosa hyperaemia** (Li, Li et al. 2013) **Hypossalivation** (Hatakka, Ahola et al. 2007)	**Salivary yeast (*C. albicans*) counts** (Hatakka, Ahola et al. 2007, Li, Li et al. 2013, Ishikawa, Mayer et al. 2014, Kraft-Bodi, Jorgensen et al. 2015, Miyazima, Ishikawa et al. 2017, Keller and Kragelund 2018)	

3. Results

In general, disease prevention (caries and periodontitis) was the objective in 52,4% (n=33) trials, while the treatment of active caries and chronic periodontitis was the goal in 7,9% (n=8) and 12,7% (n=5), respectively. Most caries trials were based on school aged children and periodontitis in adults. The studies regarding *C. albicans* usually relied on an older population, which already had higher counts of the fungi, and their objective was reducing symptoms and microbial load (n=6).

In the 28 trials that addressed caries treatment and prevention, the intervention period lasted a mean of 125 days (SD = 154), with a sample size of around 101 participants (SD=77,218), generally preschool children. Much of the sample was healthy (82%), in the sense of no active caries to treat. And so, most trials had the purpose of addressing means to prevent oral disease (82%), while only 17,9% were about treating a present caries lesion. Food products were the primary form of administration (50%), followed by tablets (21%) and lozenges (17,9%).

As for preferred strains, *L. reuteri* and *L. rhamnosus were* the choice in 21,4% (n=6) of cases, each. *L. paracasei* was employed in 14,3% (n=4) of studies. Then, regarding the probiotic's effect on oral diseases, the main results can be seen on table 5.

Table 5: Main significant (p>0.05) probiotic effects on oral ilnesses (by strain)

Probiotic strain	Anti-cariogenic effects	Periodontal effects	Anti-fungal effects
Lactobacillus rhamnosus	**Caries increment reduction in pre-school children** (Stecksen-Blicks, Sjostrom et al. 2009, Stensson, Koch et al. 2013, Rodriguez, Ruiz et al. 2016) **Reduction of *S.mutans* and/or *Lactobacillus spp.* counts** (Glavina, Gorseta et al. 2012, Juneja and Kakade 2012) **Reversal of primary root caries lesions in older adults** (Petersson, Magnusson et al. 2011) **Increased salivary buffering capability** (Villavicencio, Villegas et al. 2017)	**PI and GI reduction** (Toiviainen, Jalasvuori et al. 2014) **Reduced need for surgical treatment** (1 year follow up) (Toiviainen, Jalasvuori et al. 2014) **Reduction in the clinical manifestations (GCF, PI, GI, BoP, CAL) of periodontitis and/or gingivitis** (Alanzi, Honkala et al. 2017)	**Reduction of *C. albicans'* counts in saliva** (Hatakka, Ahola et al. 2007) **Reduction of *C. albicans'* counts in denture wearers** (Ishikawa, Mayer et al. 2014, Miyazima, Ishikawa et al. 2017)
Lactobacillus reuteri	**Reduction of *S.mutans* and/or *Lactobacillus spp*. counts** (Cildir, Sandalli et al. 2012, Alamoudi, Almabadi et al. 2018) **Risk reduction in early childhood caries** (Stensson, Koch et al. 2013, Hedayati-Hajikand, Lundberg et al. 2015)	**Reduction in the clinical manifestations (GCF, PI, GI) of peri-implantitis** (Flichy-Fernandez, Ata-Ali et al. 2015, Galofre, Palao et al. 2017) **Interleukin reduction** (Szkaradkiewicz, Stopa et al. 2014, Flichy-Fernandez, Ata-Ali et al. 2015) **Reduction in the clinical manifestations (GCF, PI, GI, BoP, CAL) of periodontitis and/or gingivitis** (Ince, Gursoy et al. 2015, Schlagenhauf, Jakob et al. 2016)	**Reduction of *C. albicans* counts in saliva and dentures** (Kraft-Bodi, Jorgensen et al. 2015)

Lactobacillus paracasei	**Reduction of *S.mutans* and *Lactobacillus spp*. counts** (Chuang, Huang et al. 2010, Teanpaisan and Piwat 2013, Wattanarat, Makeudom et al. 2015, Pahumunto, Piwat et al. 2018)		NE
Lactobacillus casei	**Reduction of *S.mutans* and *Lactobacillus* spp. counts** (Mortazavi and Akhlaghi 2012)	**Reduction of papilary bleeding and interproximal PI. Decreased MMP-3 and elastase activity and increased MPO** (Staab, Eick et al. 2009)	NE
Bacillus coagulans	**Reduction of *S. mutans* and/or *Lactobacillus spp.* counts** (Jindal, Pandey et al. 2011)	NE	NE
Lactobacillus salivarius	**Reduction of *S. mutans* and/or *Lactobacillus* spp. counts** (Nishihara, Suzuki et al. 2014) **Increased salivary buffering capacity** (Nishihara, Suzuki et al. 2014)	**Reduction in periodontal pathogens (table 3)** (Mayanagi, Kimura et al. 2009, Sajedinejad, Paknejad et al. 2017) **Reduction in the clinical manifestations (GCF, PI, GI, BoP, CAL) of periodontitis and/or gingivitis** (Sajedinejad, Paknejad et al. 2017)	NE

NE: No effect

Furthermore, 50% (n=14) of studies focused on the impacts of probiotic usage on clinical symptoms of caries progression and gingival health. Out of those, 71% showed a statistically significant ($p<0.05$) influence of the probiotic strain in use. The probiotics' capabilities to modulate oral microbiota were studied in 85% (n=24) of the trials and yielded significant results ($p<0.005$) in 60% (n=17) of the cases. Only one study looked up the influence of probiotics on immunological biomarkers.

Then, we can access the grouping of probiotic bacteria across fermentation types and its effects on the trials, as is seen on table 6.

Table 6: Probiotics grouped by type fermentation process and their statistically significant ($p<0.05$) outcomes on the trials' variables

		Significant results (%)					
		No effect	**Clinical**	**Microbiological**	**Immunological**	**More than one effect**	Total
Fermentation process	**Homofermentative**	10.0% (n=1)	-	-	-	15.4% (n=2)	5.6% (n=3)
	Facultative heterofermentative	30.0% (n=3)	61.5% (n=8)	60.0% (n=9)	33.3% (n=1)	61.5% (n=8)	53.7% (n=29)
	Heterofermentative	60.0% (n=6)	38.5% (n=5)	40.0% (n=6)	66.7% (n=2)	23.1% (n=3)	40.7% (n=22)
	Total	100.0% (n=10)	100.0% (n=13)	100.0% (n=15)	100.0% (n=3)	100.0% (n=13)	100.0% (n=54)

Lactobacilli were grouped in accordance to Table 1. *Bifidobacteria* are facultative heterofermentatives, non-lactic, acid-producing bacteria, and preparations with more than one bacterial strain with different fermentation processes were excluded to simplify the analysis. Whenever a preparation has more than one microbe, it can be unclear which one had the most (if not all) impact on the trial's outcomes. No correlation was found between fermentation process and the existence of significant results in probiotic administration. Roughly 60% of trials employing facultative heterofermentative bacteria had positive effects on both clinical and microbiological parameters (refer to table 3), and

around 40% of heterofermentative bacteria had the same results. Homofermentative bacteria were by far, the least used strains.

For the periodontitis trials the protocols lasted in average 51 days (SD= 42,15) with a sample size of 45 (SD= 18,77) volunteers. Some studies calculated sample size based on other studies with the same design (Kuru, Laleman et al. 2017, Morales, Carvajal et al. 2017), while others used convenience samples (Keller and Kragelund 2018).

In the trials addressing periodontitis, probiotics were mostly administered by oral medical appliances such as lozenges (29,6%), tablets (31%) and capsules (29%), that account for 74,1% of the analyzed trials. Food products such as cheese, yogurt and milk (8,6%) and oral hygiene appliances like toothbrushes and toothpastes (8,6%) were less used.

Most trials focused preventing periodontitis on healthy patients (37%). Most of them collected samples and performed a clinical analysis at baseline, during the usage of the probiotic, and at the end of the treatment. Some even followed the probiotic usage by a no-brush period to assess if the formulations could affect the formation of plaque and/or change the host's microbiomes.

The treatment of chronic periodontitis, characterized differently in the various studies, was the focus of 26,9% of the trials, and implant mucositis of 14,8% - the same as gingivitis (14,8%). Only one study was directed towards the study of halitosis.

Twenty-five trials studied the implications of probiotics on clinical parameters and 60% of them had at least one statistically significant ($p < 0.05$) outcome. As for the influence of beneficial microorganisms in controlling possible oral pathogens, it was addressed by 13 trials, of which 61% (8 trials) had a statistically significant ($p < 0.05$) result. Only 10 studies were based around the immunomodulation capabilities of probiotic organisms, but out of those, seven had significant ($p<0.05$) results. This indicated that probiotics such as *L. reuteri* have some capability to reduce inflammatory mediators.

As for probiotic species, the *Lactobacillus* spp. was clearly used in most studies. *L. reuteri* accounted for 30,8%, *L. rhamnosus* for 19,2% and *L. salivarius* for 15,4% of the trials. *L. reuteri*, for example, was the exclusive strain used in the clinical trials regarding implant mucositis, it also was chosen in 37,5% in periodontitis treatment trials

and 25% in gingivitis ones. *L. rhamnosus* and *L. salivarius* were both used in 22,2% of the trials regarding preventative oral health care studies.

Table 7 compiles all the major findings in this research, and table 8 regards products based on probiotic bacteria that can be purchased nowadays.

Table 7: Clinical Trials regarding probiotics and oral health care (2009 – 2019)

Reference	Intervention[3]	Sample[4]	Probiotic strain	form of administration	target disease	Outcomes (p<0.05)		
						Clinical	Microbiological	Immunological
(Stecksen-Blicks, Sjostrom et al. 2009)	105	248	*Lactobacillus rhamnosus*	Milk	Caries prevention	Yes	NT	NT
(Glavina, Gorseta et al. 2012)	14	25	*Lactobacillus rhamnosus*	Yogurt	Caries prevention	NT	Yes	NT
(Alamoudi, Almabadi et al. 2018)	28	178	*Lactobacilli reuteri*	Lozenges	Caries prevention	No	Yes	NT
(Aminabadi, Erfanparast et al. 2011)	21†	105	*Lactobacillus rhamnosus*	Yogurt	Caries prevention	NT	No	NT
(Burton, Drummond et al. 2013)	90	100	*Streptococcus salivarius*	lozenges	Caries prevention	Yes	Yes	NT
(Chuang, Huang et al. 2010)	14†	78	*Lactobacillus paracasei*	Tablet	Caries prevention	NT	Yes	NT
(Cildir, Sandalli et al. 2012)	100	19	*Lactobacillus reuteri*	Drops	Caries prevention	NT	No	NT
(Kavitha, Prathima et al. 2019)	30††	60	*Streptococcus fecalis* *Clostridium butyricum* *Bacillus mesentricus* *Lactobacillus sporogenes*	lozenge	Active caries	NT	Yes	No

[3] Total days of probiotic administration
[4] Sample at the beginning of the study

(Ghasemi, Mazaheri et al. 2017)	90†	50	*Lactobacillus acidophilus* *Bifidobacterium bifidum*	Yogurt	Caries prevention	NT	No	NT
(Gizani, Petsi et al. 2015)	510	85	*Lactobacillus reuteri*	Lozenge	Caries prevention	No	Yes	NT
(Hedayati-Hajikand, Lundberg et al. 2015)	364	138	*Streptococcus uberis,* *Streptococcus oralis ,* *Streptococcus ratti*	Chewing tablet	Caries prevention	Yes	NT	NT
(Jindal, Pandey et al. 2011)	14†	150	*Lactobacillus rhamnosus* *Bifidobacterium spp.* *Bacillus coagulans*	Sachets	Caries prevention	NT	Yes	NT
(Juneja and Kakade 2012)	21†	40	*Lactobacillus rhamnosus*	Milk	Caries prevention	NT	Yes	NT
(Ghasempour, Sefdgar et al. 2014)	14†	22	*Lactobacillus casei* *Saccharomyces cerevisiae*	Kefir drink	Caries prevention	NT	Yes	NT
(Keller, Nohr Larsen et al. 2014)	90†	36	*Lactobacillus reuteri*	Tablets	Active caries	No	NT	NT
(Nishihara, Suzuki et al. 2014)	14†	64	*Lactobacillus salivarius*	Tablets	Caries prevention	Yes	Yes	NT
(Pahumunto, Piwat et al. 2018)	90†	124	*Lactobacillus paracasei*	Milk (powder)	Caries prevention	Yes	Yes	NT
(Petersson, Magnusson et al. 2011)	450	160	*Lactobacillus rhamnosus*	Milk	Active caries	Yes	No	NT
(Rodriguez, Ruiz et al. 2016)	300	261	*Lactobacillus rhamnosus*	Milk	Caries prevention	Yes	NT	NT

(Romani Vestman, Hasslof et al. 2013)	42††	62	*Lactobacillus reuteri*	lozenges	Caries prevention	NT	Yes	NT
(Mortazavi and Akhlaghi 2012)	14	60	*Lactobacillus casei*	Cheese	Caries prevention	NT	Yes	NT
(Singh, Damle et al. 2011)	10†	40	*Bifidobacterium lactis* *Lactobacillus acidophilus*	Ice cream	Caries prevention	NT	Yes	NT
(Stensson, Koch et al. 2013)	364††	113	*Lactobacillus reuteri*	Oil drops (both)	Caries prevention	Yes	No	NT
(Taipale, Pienihakkinen et al. 2012)	30††	106	*Bifidobacterium animalis*	Tablets (on spoon/pacifie)	Caries prevention	NT	Yes	NT
(Teanpaisan and Piwat 2013)	28†	40	*Lactobacillus paracasei*	Milk powder	Caries prevention	NT	Yes	NT
(Villavicencio, Villegas et al. 2017)	270	363	*Lactobacillus rhamnosus* *Bifidobacteruim longum*	Milk	Preventive oral care	Yes	No	NT
(Wattanarat, Makeudom et al. 2015)	364	60	*Lactobacillus paracasei*	Milk	Preventive oral care	Yes	Yes	Yes
(Flichy-Fernandez, Ata-Ali et al. 2015)	30††	77	*Lactobacillus reuteri*	Tablets	Peri-implant mucositis	Yes	NT	Yes
(Galofre, Palao et al. 2017)	30†	44	*Lactobacillus reuteri*	lozenge	Peri-implant mucositis	Yes	No	NT

(Ince, Gursoy et al. 2015, Meenakshi, Gupta et al. 2016)	21††	55	*Lactobacillus reuteri*	lozenge	Chronic periodontitis	Yes	NT	NT
(Hallstrom, Lindgren et al. 2015)	90†	49	*Lactobacillus reuteri*	lozenge	Peri-implant mucositis	No	No	No
(Iwasaki, Maeda et al. 2016)	12†	39	*Lactobacillus plantarum*	Capsule	Chronic periodontitis	Yes	NT	NT
(Morales, Gandolfo et al. 2017)	90††	47	*Lactobacillus rhamnosus*	Tablets	Chronic periodontitis	No	No	No
(Alkaya, Laleman et al. 2016)	56	40	*Bacillus subtilis* *Bacillus megaterium-Bacillus pumulus*	Toothpaste, mouth rinse and tooth brush	Generalized gingivitis	No	NT	NT
(Alanzi, Honkala et al. 2017)	28	101	*Lactobacillus rhamnosus* *Bifidobacterium lactis*	lozenge	Periodontitis prevention	Yes	yes	NT
(Tobita, Watanabe et al. 2018)	28	16	*Lactobacillus crispatus*	Food tablet	Periodontitis prevention	Yes	yes	NT
(Harini and Anegundi 2010)	14	45	*No info*	Mouth rinse	Periodontitis prevention	Yes	NT	NT
(Kuru, Laleman et al. 2017)	28	51	*Bifidobacterium animalis*	Yogurt	Periodontitis prevention	Yes	NT	Yes

(Iwamoto, Suzuki et al. 2010)	28	20	*Lactobacillus salivarius*	Tablets	Halitosis	Yes	yes	NT
(Keller, Brandsborg et al. 2017)	28	47	*Lactobacillus rhamnosus* *Lactobacillus curvatus*	Tablets	Gingivitis	No	NT	No
(Laleman, Yilmaz et al. 2015)	168	48	*Streptococcus oralis KJ3, Streptococcus uberis KJ2, Streptococcus ratti JH145*	Tablets	Chronic periodontitis	No	No	NT
(Lee, Kim et al. 2015)	14	34	*Lactobacillus brevis*	lozenge	Periodontitis prevention	No	NT	Yes
(Mayanagi, Kimura et al. 2009, Macura-Karbownik, Chladek et al. 2016)	56	66	*Lactobacillus salivarius*	Tablets (dissolving)	Periodontitis prevention	NT	Yes	NT
(Mongardini, Pilloni et al. 2016)	14	20	*Lactobacillus plantarum* *Lactobacillus brevis*	Tablets	Periodontitis prevention (implants)	Yes	NT	NT
(Montero, Iniesta et al. 2017)	42	59	*Lactobacillus plantarum* *Lactobacillus brevis* *Pediococcus acidilactici*	Tablets	Gingivitis	No	Yes	NT
(Morales, Carvajal et al. 2017)	90††	28	*Lactobacillus Rhamnosus*	Sachet	Chronic periodontitis	No	NT	NT

(Sajedinejad, Paknejad et al. 2017)	28	45	*Lactobacillus salivarius*	Mouth rinse	Chronic periodontitis	Yes	Yes	NT
(Schlagenhauf, Jakob et al. 2016)	49	45	*Lactobacillus reuteri*	lozenge	pregnancy gingivitis	Yes	NT	Yes
(Shimauchi, Mayanagi et al. 2008)	56.0	66	*Lactobacillus salivarius*	Tablets	Periodontitis prevention	Yes (smokers)	NT	Yes (smokers)
(Staab, Eick et al. 2009)	56.0	50	*Lactobacillus casei*	Milk	Periodontitis prevention	No	NT	Yes
(Szkaradkiewicz, Stopa et al. 2014)	-	24	*Lactobacillus reuteri*	Tablets (suction)	Chronic periodontitis	Yes	NT	Yes
(Tada, Masaki et al. 2017)	168	30	*Lactobacillus reuteri*	Tablets	Peri implant mucositis	Yes	Yes	NT
(Teughels, Durukan et al. 2013)	84	30	*Lactobacillus reuteri*	lozenge	Chronic periodontitis	No	Yes	NT
(Toiviainen, Jalasvuori et al. 2014)	28	62	*Lactobacillus rhamnosus Bifidobacterium animalis*	lozenge (chewing gum)	Periodontitis prevention	Yes	No	NT
(Hatakka, Ahola et al. 2007)	112	294	*Lactobacillus rhamnosus Propionibacterium*	Cheese	*Candida albicans* infection	Yes	Yes	NT
(Ishikawa, Mayer et al. 2014)	35	59	*Lactobacillus rhamnosus Lactobacillus acidophilus,*	Capsule	*Candida albicans* infection	NT	Yes	NT

			Bifidobacterium bifidum					
(Keller and Kragelund 2018)	112 †	22	*Lactobacillus reuteri*	lozenges	*Candida albicans* infection and lichen planus	Yes	No	NT
(Li, Li et al. 2013)	28	65	*Bifidobacterium Longum* *Lactobacillus bulgaricus* *Streptococcus thermophilus*	lozenges	*Candida* associated stomatitis	No	Yes	NT
(Miyazima, Ishikawa et al. 2017)	56	60	*Lactobacillus acidophilus* *Lactobacillus rhamnosus*	Cheese	*Candida albicans* infection	NT	Yes	NT
(Kraft-Bodi, Jorgensen et al. 2015)	84	219	*Lactobacillus reuteri*	lozenge	*Candida albicans* infection	No	Yes	NT
(Sanctis, Belgoia et al. 2019)	Variable*	75	*Lactobacillus brevis CD2*	lozenges	Oral mucositis (cancer therapy side effect)	No	No	NT

† Follow up: less than 6 months after intervention period
†† Follow up: 6 months or more after intervention period
NT – parameter not tested in the trial
Candida albicans infection – high C. albicans counts
(*)probiotic administration was concomitant with radiotherapy treatment – RT - (and a week after RT) and variable for each patient

Table 8: Commonly used probiotic products

Brand/ Product	Strain	Posology	Significant (p<0.05) results
BioGaia Prodentis lozenges / (Gum) Periobalance†	*Lactobacillus reuteri* Prodentis (*L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289) $1x10^8$ CFU 30 Probiotic lozenges (24 g)	1 – 2 lozenges a day (let the lozenges melt in the mouth, after brushing)	PD and CAL reduction, as well as in pro inflammatory cytokines (Szkaradkiewicz, Stopa et al. 2014) Improvement of PD and CAL when used in junction with professional prophylaxis (Teughels, Durukan et al. 2013) Improvements on clinical parameters of peri-implantits (Flichy-Fernandez, Ata-Ali et al. 2015, Galofre, Palao et al. 2017) Reduction of GI and PI in pregnancy gingivitis (Schlagenhauf, Jakob et al. 2016) Reduction in *S. mutans* counts in children (Alamoudi, Almabadi et al. 2018)
Wakamoto Pharmaceutical Co. Minna No zendamakin W21 tablets	*Lactobacillus salivarius* $6.7x10^8$ CFU + Xilitol (280 mg)	1 – 2 lozenges a day (let the tablets melt in the mouth)	Improvement of physiological halitosis (Iwamoto, Suzuki et al. 2010) Reduction in periodontal pathogens (Mayanagi, Kimura et al. 2009) Improvement of periodontal health in smokers (Shimauchi, Mayanagi et al. 2008) Reduction in *S. mutans* in children (Nishihara, Suzuki et al. 2014)
Honsha Co, Ltd Yakult	*Lactobacillus casei shirota* $1x10^6$ CFU	Fermented milk product (one daily bottle)	Reduction in induced plaque formation (Slawik, Staufenbiel et al. 2011) MMP-3 reduction (Staab, Eick et al. 2009)

† Commercially available in Portuguese pharmacies

4. Discussion

This bibliographic revision has shown that probiotics have proven clinical benefits in many areas within the scope of action dentistry and oral medicine. The most prevalent findings regarded the efficiency of certain probiotic strains in avoiding cavity lesions in children, as well as reducing periodontal disease symptoms. This was due mostly to the reduction in the proliferation of cariogenic and periodontal pathogens. Nevertheless, there are various nuances in these processes that need to be addressed.

Lactobacilli can be both a risk marker, isolated in healthy mouths, and a caries prevention method, used in probiotic preparations. While some species tend to appear in deep caries, corelated with the lesion's progression, other species have been shown to be able to help modulate the microbial environment around them. For example, a study points out that *L. fermentum* and *S. mutans* with *S. sobrinus* were positively associated with caries, while the probiotic *L. acidophilus* was negatively associated with caries in preschool aged children (Kanasi, Johansson et al. 2010). Even so, the production of lactic acid from beneficial species can be considered as a side effect of their usage. *Lactobacilli* can potentially be cariogenic, but account for a very small percentage of the oral microbiome and have a low impact in the development of caries – even though they have a more significant role in its evolution, across the cavitated phase (Lahtinen, Salminen et al. 2012). Both xylitol and fluoride have also been used to successfully prevent caries lesions in children, but their administration can also result in the development of fluoride resistant bacteria (Marinho, Worthington et al. 2013, Banerjee, Sengupta et al. 2016, Lin, Fang et al. 2016).

Whenever *Lactobacillus* counts are evaluated in these trials, the strain type is important since the increase in probiotic lactobacilli may be beneficial (testing the persistence of the probiotic after the intervention period) while other species within the genus can be detrimental (cavitated lesions). For example, *L. plantarum* can quickly transform sugars in to lactic acid, while *L. paracasei* and *L. rhamnosus* have a slower metabolism, being less cariogenic (Lahtinen, Salminen et al. 2012). None of the trials evaluated in this study have employed *L. plantarum* to treat or prevent oral cavities. Another study showed that *L. reuteri* had the capability to reduce the growth of cariogenic *S. mutans* but it wasn´t always detected in the mouth after the intervention period (Romani Vestman, Hasslof et al. 2013). Even the administration of probiotics, as early as at birth or infancy, could effectively reduce *S. mutans* counts throughout childhood, with positive

effects on primary dentition (Stensson, Koch et al. , Taipale, Pienihakkinen et al. 2013). These probiotics are intentionally administered and can be more effective if they are given the chance to colonize the oral biofilm earlier (Lahtinen, Salminen et al. 2012). In these cases, while pathogens are being effectively reduced for years, no traces of the probiotic strain are found in recent saliva samples. Hence, the effects of early usage of probiotics in children are long lasting, but the colonization itself isn't – meaning, the microbes do not definitely colonize the mouth. Maybe by colonizing plaque in its formation, pathological microbes aren't allowed to adhere.

As most studies regarded caries prevention and progression on children, the preferred method of probiotic administration tended to be food products. Food products have high oral clearance and so, measures need to be taken in order to keep them longer in the mouth. Some studies refer giving specific recommendations to the patients taking probiotic milk: to drink it slowly, in portions, without heating it up and avoiding brushing their teeth for up to 1 hour (Juneja and Kakade 2012). Others also point out the need to wait 1 hour before brushing, after taking a kefir drink (Ghasempour, Sefdgar et al. 2014).

As for the effects on microbiome modulation, the administration of probiotic bacteria tends to have different effects on streptococci and on lactobacilli. A trial found that a combination of *Bifidobacterium lactis* and *L. acidophilus* successfully decreased *S. mutans* colonization but had no effect on other *Lactobacillus* strains (Singh, Damle et al. 2011). *L. casei* showed a similar behavior (Mortazavi and Akhlaghi 2012). On the other hand, *L. reuteri* showed to have the capability to reduce other *Lactobacillus* strains on more than one study (Gizani, Petsi et al. 2015, Alamoudi, Almabadi et al. 2018). And *L. paracasei* was able not only to suppress the growth of MS and other lactobacilli, but did so while producing less lactic acid than other strains – more cariogenic strains, such as *L. salivarius* (Wattanarat, Makeudom et al. 2015). In fact, *L. salivarius* was never used on its own as a probiotic strain to address caries in any of the presented trials.

Different stages of caries progression are related with different pathogens – *S. mutans* in early lesions and *Lactobacillus* in advanced ones. And different strains of *lactobacilli* showed to have capability to reduce the pathogenic microbes of both phases. It is also important to note that lactobacilli, as lactic acid producers are potentially cariogenic, being widely present in carious dentine (Byun, Nadkarni et al. 2004). That may be the reasoning behind the usage of these species in prevention of carious lesions

instead of in its treatment. Remineralization attempts with probiotics were generally unsuccessful. Most trials in this study revolved around preventing caries in children. Hence the usage of acid producing bacteria that can be added to amenable food products such as milk, cheese and ice cream.

In the periodontitis trials, health and disease are measured in different manners. A study defines moderate to severe periodontitis as PD > 4 mm, CAL > 3 mm and bone loss > 3 mm, while another describes periodontitis as patients with detected horizontal bone loss, the presence of at least 2 teeth with an approximal site each with a PD of 5-7 mm and a GI of ≥2 in each quadrant (Ince, Gursoy et al. 2015, Sajedinejad, Paknejad et al. 2017). Furthermore, some trials specify periodontitis as moderate or severe, according to probing depths and other clinical parameters. As recently as 2011, the American Academy of Periodontology and the European Federation of periodontology came up with a new *Classification for Periodontal and Per-Implant diseases and Conditions*, rendering the concepts of chronic and aggressive periodontitis obsolete. The trials in this study do not comply by a standardized definition of periodontal illness, and so their results are not directly comparable.

Different strains of *L. salivarius* can be more or less effective according to their probiotic features (Ruiz, Margolles et al. 2013, Sajedinejad, Paknejad et al. 2017). Sajedinejad et all in their 2017 clinical trial found that *L. salivarius* NK02 had the highest microbial activity against *A. actinomycetemcomintans* in addition to all the other parameters listed before. While these are beneficial it is important to note that due to the high oral clearance, the local application of probiotics would be of little effect. Nevertheless, the immunomodulation caused by these species in the GI tract may positively impact the oral cavity. Other probiotic products such as lozenges, chewing gum and straws may prove to be more effective than mouthwashes and food items for these reasons (Charalampopoulos and Rastall 2009). And 74,1% of the trials analyzed administered the probiotics as lozenges, tablets or capsules. Some studies even went as far as explaining if these devices were to be left to dissolve in the mouth (Hallstrom, Lindgren et al. 2015, Galofre, Palao et al. 2017, Tobita, Watanabe et al. 2018) or simply consumed (Iwasaki, Maeda et al. 2016).

According to J. H. Meurman (Charalampopoulos and Rastall 2009) *Lactobacillus spp.* have varying antimicrobial activity across its different strains. Different pathogens

may need the action of a different probiotic strain. *L. reuteri* inhibits the growth of *P. gingivalis* and *P. intermedia* in 82 and 55%, respectively, with that diminishing gingival bleeding (Charalampopoulos and Rastall 2009). And, in the present clinical trial review, *L. reuteri* was also proven to be effective against *P. gingivalis*. *L. rhamnosus* has shown evidence to be efficient at reducing the levels of *A. actinomycetemcomitans* and *F. nucleatum* in saliva and plaque, and *P. gingivalis* in plaque (Alanzi, Honkala et al. 2017). *L. salivarius* decreased the counts of A. *actinomycetemcomitans* and *T. forsythia*. Homofermentative lactobacilli were more frequent in healthy mouths, in comparison with chronic periodontitis patients. Nevertheless, both homofermentative and heterofermentative probiotics have positive effects on biofilm modulations, even though the complete mechanisms behind this dynamic are still unknown (Lahtinen, Salminen et al. 2012). A study found that the strongest anti-microbial activity was seen in facultative heterofermentative bacteria and strict homofermentatives. While *L. gasseri* and *L. crispatus* (homofermentatives) showed to highly inhibit *P. gingivalis*, *L. plantarum* (heterofermentative) had no impact on periodontal pathogens. In low glucose environments microbial activity decreased due to the reduction of fermentation substrate and lower lactic acid production (Koll-Klais, Mandar et al. 2005). It is important to note that most of the studies that were performed on patients with periodontitis, the usage of probiotics was concomitant with mechanic professional prophylaxis. No studies were performed where a control group had no prophylaxis done, for obvious ethical reasons. Probiotics were evaluated as coadjutant to planning and root scaling, the gold standard of non-surgical periodontal treatment. Whenever the effect of probiotics on their own was tested, healthy patients (after a period of probiotic products intake), were asked to stop oral health hygiene for a small period. This provoked intentional inflammation and the first stages of plaque formation. In this matter, 3 studies were able to prove that the regular usage of probiotic supplements could diminish the counts of oral periodontal pathogens (Mayanagi, Kimura et al. 2009, Alanzi, Honkala et al. 2017, Tobita, Watanabe et al. 2018), and one showed that they didn't (Toiviainen, Jalasvuori et al. 2014). Other than controlling bacterial populations, probiotics can also stimulate and regulate the immune system. Gill, Grover et al. (Charalampopoulos and Rastall 2009) refer that, among other functions, probiotics can increase cellular immunity (NK cell activity, phagocytosis and oxidative bursts), humoral activity (increase in immunoglobulin levels – IgA, IgG, IgM) and interfere with the production of inflammatory cytokines (Charalampopoulos and Rastall 2009). *L. reuteri* was pointed as capable of reducing inflammatory cytokine levels

in three trials (Szkaradkiewicz, Stopa et al. 2014, Flichy-Fernandez, Ata-Ali et al. 2015, Schlagenhauf, Jakob et al. 2016). Finally, the most studied variables were the clinical parameters – GI, PD, BoP and PI – in 24 trials. *L. reuteri* (n=7) and *L. rhamnosus* (n=5) were the most used probiotic strains. 60% of all the studies considering these variables had a positive outcome.

Taking together the above-described information, the best probable usage of probiotics in the treatment of periodontal illnesses is as an aid to home oral hygiene and professional prophylaxis.

Mucositis has been mostly approached in these recent trials as an implant related disease. In this manner it is highly correlated with the maintenance of periodontal health, and hence generally circumscribed localized issue.

Some studies refer the importance of non-surgical, mechanic periodontal treatment, before initiating probiotics treatment, in order to reduce the bacterial load pretrial and ensure the best results (Hallstrom, Lindgren et al. 2015, Mongardini, Pilloni et al. 2016, Galofre, Palao et al. 2017). These trials aimed at preventing the development of peri implant mucositis.

Other trials have the objective of treating active implant mucositis. Hence, they don't include healthy individuals or patients who used antibiotics 3 months prior to the study (Hallstrom, Lindgren et al. 2015). While others specifically select patients with <15% full mouth plaque score and <15% full mouth bleeding score. After a phase of intentional plaque induction at the implant site (14 days, using an acrylic stent during self-performed oral hygiene), the probiotic test protocol was put to the test (Mongardini, Pilloni et al. 2016). These recent trials have shown that probiotics seem to have little to no influence pathological periodontal microbiomes in crevicular gingival fluid. Only one study found that *L. reuteri* had a significant on the bacterial load of *P. gingivalis* in peri-implant mucositis, while it had no other impacts on the remaining bacteria. *A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola* and *P. intermedia*, major periodontal pathogens from the red and yellow group (gram negative, facultative anaerobic or complete anaerobes) were unaffected (Galofre, Palao et al. 2017). Even older studies have found no connections between mucositis and probiotics usage (Flichy-Fernandez, Ata-Ali et al. 2015). However, there seems to be a positive effect on the usage of *L. reuteri:* reduced levels of inflammatory mediators in crevicular gingival fluid.

Nevertheless, it is shown that the best results in managing peri implant health can be achieved with proper oral hygiene and professional mechanical removal of dental plaque. In these instances, the usage of probiotics may not be strictly recommended solely on a cost effectiveness basis.

As for mucositis as sequelae of oropahringeal cancer treatment, it is generally accepted that it is associated with the intensity and toxicity of both radio and chemotherapy. The cytotoxicity of these treatments has direct effects on connective tissue and epithelial cells, resulting of thinning of the epithelium and, as time progresses, it's loss. On such studies measurements other than crevicular fluid are used, such as the oral mucositis grade (OM). The OM is a clinical observation measure that ranges between 0 and IV, from the least amount of oral discomfort and mucosal compromise (0) to the greatest (IV). These studies have, due to these variables, more difficulties in drawing definitive conclusions.

In neutropenic patients with mucositis, there is an increased risk for systemic infections originating from opportunistic elements of the oral microbiome due to mucosal ulceration. In that sense there is an increased importance in avoiding the proliferation of oral pathogens in these immunocompromised individuals (Greenberg, Glick et al. 2008). A recent trial attempted to modulate the microbial composition of the saliva of patients with neck and head tumors, by adding a strain of *L. brevis* into their diet. No differences were observed between the placebo control group (sodium bicarbonate mouthwash) and the group receiving the probiotic (Sanctis, Belgoia et al. 2019). On the other hand, it was found that the usage lozenges containing *L. brevis* reduced the development of grade III and IV mucositis (28% of patients treated with *L. brevis* did not develop mucositis, while only 7% of those on the placebo had the same outcome) (Sharma, Rath et al. 2012). One must note a difference in metrics between these studies: while one assessed a clinical parameter (mucositis grade), the other discussed the effects on the microbiome. Probiotics seem to have a positive influence on the patient's quality of life, but the underlying biological mechanisms need further research. For example, the positive results in reducing the production of cytokines cited in other trials (Staab, Eick et al. 2009, Szkaradkiewicz, Stopa et al. 2014, Flichy-Fernandez, Ata-Ali et al. 2015, Kuru, Laleman et al. 2017), has been proven beneficial. Even though, there might not be a direct effect in pathogen control, probiotics may help strengthen the mucosal barrier by reducing inflammatory molecules that negatively impact epithelial cell proliferation and worsens

tissue damage (Greenberg, Glick et al. 2008). Furthermore, there is a difference between trying to modulate the microbiome of a healthy individual - cases of peri-implantitis - versus the one existing on a patient during cancer treatment - mucositis due to cancer treatment toxicity.

As for the efficiency of probiotics in the treatment of yeast infections, it is measured in comparison with the one already achieved by anti-fungal medications. Probiotics have the added benefit on not causing microbial resistance and being generally less aggressive to the host's organism. Li et all (2013) prove that adding a probiotic to nystatin increases the reduction in *C. albicans* colonization, versus the nystatin monotherapy. A study that compared the two separately, would be of interest. Another study directly compared the effects of *L. reuteri* and nystatin as prophylaxis in skin and stool *Candida* colonization in very low birth weight infants. In this study the *L. reuteri* was as effective as nystatin. The skin samples were collected from the axilla, interinginous and moist mucosa region, which points the fact that the application of this protocol to the oral cavity might be a viable research option (Oncel, Arayici et al.).

Probiotic effects are strain specific, therefore there is a need to test which strains are more suited to treat a specific condition. An investigation tested *L. acidophulus* and *L. rhamnosus* in their capabilities to reduce *Candida spp.* infections, and both were effective (Ishikawa, Mayer et al. 2014, Miyazima, Ishikawa et al. 2017). It is suggested that to assess the varying impacts of both strains, a larger sample and longer evaluation period would be necessary. Another study tested the anti-fungal capabilities of *L. rhamnosus* and *L. casei* on resin surface dentures. Both strains were effective at reducing yeast proliferation and did not affect the roughness of the resin, an added benefit for patients that use removable oral prosthetics (Song and Lee 2017).

Probiotic delivery vehicles also need to be addressed. Food products such as cheese and milk have a shorter activity clearance due to salivary flow. Direct application on oral prosthetics or a more viscous adherent vehicle could be beneficial (Ishikawa, Mayer et al. 2014).

Medical co-mobilities such as diabetes and medication intake should also be considered, especially in studies regarding elderly populations. Diabetes, generally regarded as a *Candida spp.* colonization facilitator (due to reduced salivary flow), had no impact in the probiotic's effect (Ishikawa, Mayer et al. 2014).

Regarding the treatment of head and neck tumors, sequelae such as xerostomia and, therefore, oral mucositis and candidiasis may arise. While no specific trials on the direct usage of probiotics on this population, it is safe to infer that maintaining and adequate salivary flow and controlling the proliferation of potentially pathogenic fungi would be of great advantage. So, besides the standard preventative measures (diet control, fluoride supplementation, treatment of infectious sites and regular oral prosthetic's maintenance), the cancer patient can also benefit from the usage of probiotic preparations in order to avoid a range of oral diseases: caries, periodontal disease, xerostomia and mucositis.

As it has been discussed before, there seems to be an association between lichen planus and *C. albicans* infections (Neville, Damn et al.). Hence the attempt to tackle both conditions with the same probiotic microorganism is justifiable. The usage of *L. reuteri* has only had significant effects in the decrease of the gingival index (GI), but no effects in *C. albican's* counts (Keller and Kragelund 2018). It is believed that oral microbes may also be implied in the progression of lichen planus. A study found that patients with current Lichen planus had relatively higher counts of *Porphyromonas* and *Solobacterium*, in comparison with healthy controls (Wang, Lu et al. 2016). *Porphyromonas* is especially prone to generate inflammatory response and cytokine production. Therefore, the improvement of gingival index measures may prove beneficial to control the proliferation of *Porphyromonas* and help reduce inflammation and pain.

4.1. Currently available commercial probiotic formulations

The *Lactobacillus prodentis*® (*L. reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 - $1x10^8$ CFU) formulation is commonly used across studies. While it shows positive results in reducing periodontal disease symptoms, it is less effective in reducing its pathogens. However, when applied to the treatment of caries in children, it has shown the ability to suppress the growth of *S. mutans* in the study by Alamoudy, Almabady et all (2018). Nevertheless, this product has also produced some non-significant results: no microbiome alterations (reduction of *S. mutans*) (Gizani, Petsi et al. 2015), as well as no effect on the surgency of white spot lesions (Keller, Nohr Larsen et al. 2014, Gizani, Petsi et al. 2015). BioGaia also produces oil drops, mostly aimed at the regulation of gut microbiota (*Lactobacillus protectis* ® - L. reuteri DSM 17938).

These products have originated from *a L. reuteri* strain isolated from breast milk in the 1950's - ATCC 55730. This strain was used in the oil drops formula applied by Stensson, Koch et al (2013) on their clinical trial. The test group, 60 (out of 113) mothers were given daily probiotic drops during the 4 weeks before the expected date of delivery, and their children for 365 days (their first year of life). Nine years after the intervention, children in the test group had reduced caries prevalence and gingivitis score in primary dentition (Stensson, Koch et al. 2013). Hence, this product seems particularly suitable to treat periodontitis symptoms and to prevent the surgency of caries in primary dentition, if given to children early on in life. Periobalance ® is available in Portuguese pharmacies. A possible clinical application of *L. reuteri* to periodontal disease treatment can be the daily intake of probiotic lozenges after scaling and root planning. The most common approach is the usage of chlorohexidine mouth rinses during a controlled period after SRP. Chlorohexidine is still the gold standard when it comes to periodontal disease treatment because it performs three different tasks simultaneously: it is both a bactericide, a bacteriostatic and has substantivity in the oral cavity. This cannot be said about probiotics, whose presence in the oral cavity is short lived. Nevertheless, there is no evidence pointing that probiotics have the same side effects as chlorohexidine, such as extrinsic teeth staining (Moshrefi 2002), and less frequently, mucosal desquamation and subjective feelings of dryness, soreness or burning sensation (Flotra 1973). Teeth staining, was more prevalent as usage period of chlorohexidine increased (Tartaglia, Tadakamadla et al. 2019). Furthermore, chlorohexidine is considered as a pollutant, being found in hospital sewage waters (Lasek, Karpel et al. 2018) and is suggested to be cytotoxic towards osteoblastic, endothelial and fibroblastic cell lines in "in vitro" studies (Giannellia, F.Chellinib et al. 2008, Reddersen, Wiegand et al. 2019). In this sense, probiotics can be an option when long term management of periodontitis is concerned.

The W21 tablets produced by Wakamoto Pharmaceutical Co were effective at controlling periodontal (Mayanagi, Kimura et al. 2009) and cariogenic pathogens (Nishihara, Suzuki et al. 2014). They were also capable of improving periodontal health in smokers and reducing physiological halitosis (Shimauchi, Mayanagi et al. 2008, Iwamoto, Suzuki et al. 2010). Despite the positive results, these products aren't, at the moment, available in Portugal.

The *L. casei* Shirota found in Yakult ® yogurts has shown to be effective at reducing plaque formation and gingival inflammatory markers (Staab, Eick et al. 2009,

Slawik, Staufenbiel et al. 2011). This product is mostly associated with gastrointestinal benefits but *L. casei* and *L. paracasei* strains have also been proved to have positive effects on oral health, especially on caries prevention. An example is the novel *L. paracasei* SD1 (Teanpaisan and Piwat 2013, Wattanarat, Makeudom et al. 2015). However, these microorganisms haven't been added to commercially available formulations yet.

5. Conclusions

Probiotics have proven to be beneficial in preventing the development of cavities in school aged children, reducing inflammation markers and clinical symptoms of periodontitis in adults and fungal counts in the mucosa of the elderly.

While most studies show that there is some benefit in the usage of probiotics to ameliorate the most prevalent conditions seen in the dentist's daily practice, their effects aren't completely predictable and hence they shouldn't be used in a monotherapy regime.

6. Future research developments

There is still room for further research, mainly in realm of the possible benefits that probiotic usage can have on some populations, namely patients receiving treatment for head and neck cancer, as well as some immunologically mediated illnesses with oral manifestations like lichen planus, pemphigus, and aphthous stomatitis. In the end, research proves that probiotics are a clinically verified treatment option and can safely and effectively be used in many oral aliments and in all age groups.

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PARECER DO ORIENTADOR

(Entrega do trabalho final de monografia)

Informo que o trabalho de Monografia desenvolvido pela estudante Cláudia Sofia da Silva Campos com o título: Probióticos em Medicina Dentária e Oral: tendências recentes/ Probiotics in dentistry and oral medicine: recent trends, está de acordo com as regras estipuladas pela FMDUP, e foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

11/05/2020

A orientadora

FACULDADE DE MEDICINA DENTÁRIA DA UNIVERSIDADE DO PORTO

DECLARAÇÃO

Monografia de investigação

Declaro que o presente trabalho, no âmbito da Monografia de investigação, integrado no MIMD, da FMDUP, é da minha autoria e todas as fontes foram devidamente referenciadas.

11/05/2020

Cláudia Sofia da Silva Campos

A investigadora