To manage **Oral Dysbiosis** in **Gingivitis, Periodontitis,** & **Dental Caries**

SPORLAC®-DG

Lactobacillus paracasei **GMNL-33 Tablet**

DOSAGE

1 Tablet Daily Before Bedtime.

DIRECTIONS FOR USE

Place the Tablet on the Tongue until Dissolved. Not to be Swallowed.

India's First Probiotic* for Dental Health

*Heat-killed probiotic

JB/YUVA/SPOR DG /Q1/PM/18/JL/JS/FY 25-26

For the use of Registered Medical Practitioner, or a Hospital or a Laboratory only

SPORLAC® - DG

PRODUCT MONOGRAPH

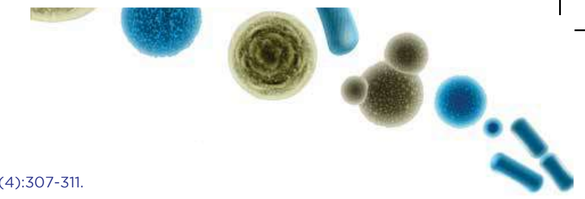
Back Page

Closeed Size: 14.8 Inch x 10.5 Inch

Front

SPORLAC® - DG

CONTENT INDEX

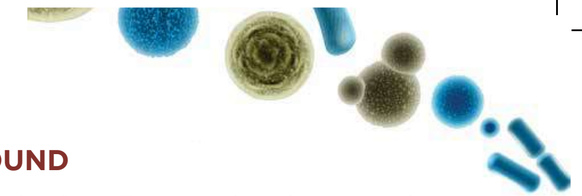


36. Chuang LC, *et al.* Clin Oral Investig. 2011; 15(4): 471–476.
37. Srinivasan S, *et al.* J Indian Soc Pedod Prev Dent. 2017 Oct-Dec;35(4):307-311.
38. Arat Maden E *et al.* Oral Health Prev Dent. 2017;15(2):117-121.
39. Maden EA, Altun C, Ozmen B, Basak F. Antimicrobial Effect of Toothpastes Containing Fluoride, Xylitol, or Xylitol-Probiotic on Salivary Streptococcus mutans and Lactobacillus in Children. Niger J Clin Pract 2018;21:134-8.
40. Data on file.
41. Ishikawa, H., *et al.* (2003). "Probiotic bacteria reduce mutans streptococci levels in the human oral cavity." *Journal of Applied Microbiology,* 95(2), 344-352.
42. Chen, Y., *et al.* (2020). "The anti-inflammatory effects of Lactobacillus paracasei GMNL-33 in the oral environment." *Journal of Functional Foods,* 68, 103882.
43. Filoche, S., *et al.* (2010). "Probiotics and oral health effects in a long-term periodontitis model." *Journal Of Clinical Periodontology,* 37(2), 195-203.
44. Kanasi, E., *et al.* (2010). "Microbial communities in an aging population." *Proceeding of the National Academy of Sciences,* 107(Supplement_1), 224-229.
45. Salminen, S., *et al.* (2018). "Postbiotics: A novel approach to enhance the efficacy and safety of probiotics. *"Trends in Food Science & Technology,"* 83, 26-34.
46. Plaza-Diaz, J., *et al.* (2019). "Safety and tolerance of postbiotics in health applications." *Nutrients,* 11(2), 297.

Closeed Size: 14.8 Inch x 10.5 Inch

References

1. Exp Biol Med (Maywood). 2023 Sep 9;248(15):1288–1301. doi: 10.1177/15353702231187645
2. Microorganisms. 2023 May 31;11(6):1453. doi: 10.3390/microorganisms11061453
3. Bostanghadiri N, Kouhzad M, Taki E, Elahi Z, Khoshbayan A, Navidifar T and Darban-Sarokhalil D (2024) Oral microbiota and metabolites: key players in oral health and disorder, and microbiota-based therapies. *Front. Microbiol.* 15:1431785. doi: 10.3389/fmicb.2024.1431785
4. Kilian, M., Chapple, I., Hannig, M. *et al.* The oral microbiome – an update for oral healthcare professionals. Br Dent J 221, 657–666 (2016). https://doi.org/10.1038/sj.bdj.2016.865
5. Scannapieco FA, Dongari-Bagtzoglou A. Dysbiosis revisited: Understanding the role of the oral microbiome in the pathogenesis of gingivitis and periodontitis: A critical assessment. *J Periodontol*. 2021;92(8):1071-1078. doi:10.1002/JPER.21-0120
6. Microorganisms. 2023 May 31;11(6):1453. doi: 10.3390/microorganisms11061453
7. Abdulkareem, A. A., Al-Taweel, F. B., Al-Sharqi, A. J. B., Gul, S. S.,Sha, A., & Chapple, I. L. C. (2023). Current concepts in the pathogenesis of periodontitis: from symbiosis to dysbiosis. *Journal of Oral Microbiology*, 15(1). https://doi.org/10.1080/20002297.2023.2197779
8. *Microorganisms* 2024, 12(9), 1797; https://doi.org/10.3390/microorganisms12091797
9. Cugini C, Ramasubbu N, Tsiagbe VK and Fine DH (2021) Dysbiosis From a Microbial and Host Perspective Relative to Oral Health and Disease. *Front. Microbiol.* 12:617485. doi: 10.3389/fmicb.2021.617485
10. Clin Oral Investig. 2024 May 10;28(6):306. doi: 10.1007/s00784-024-05687-2
11. *Microorganisms* 2024, 12(9), 1797; https://doi.org/10.3390/microorganisms12091797
12. Bostanghadiri N, Kouhzad M, Taki E, Elahi Z, Khoshbayan A, Navidifar T and Darban-Sarokhalil D (2024) Oral microbiota and metabolites: key players in oral health and disorder, and microbiota-based therapies. *Front. Microbiol.* 15:1431785. doi: 10.3389/fmicb.2024.1431785
13. https://www.who.int/news-room/fact-sheets/detail/oral-health#:~:text=The%20WHO%20Global%20Oral%20Health,health%20conditions%20and%20other%20NCDs.
14. https://onlinelibrary.wiley.com/doi/pdf/10.1111/odi.14516
15. Future Sci OA. 2023 Jun 12;9(8):FSO856. doi: 10.2144/fsoa-2023-0062
16. Thomas, C., Minty, M., Vinel, A., Canceill, T., Loubières, P., Burcelin, R., Kaddech, M., Blasco-Baque, V., & Laurencin-Dalicieux, S. (2021). Oral Microbiota: A Major Player in the Diagnosis of Systemic Diseases. *Diagnostics,* 11(8), 1376. https://doi.org/10.3390/diagnostics11081376
17. Zhu, B., et al. (2018). "Antibiotic resistance in the oral cavity: microbiome adaptations and implications." *Current Opinion in Microbiology,* 47, 9-15.
18. World Health Organization (WHO). (2020). "Antimicrobial resistance." https://www.who.int/
19. *Mariana B Rebelo,* Cláudia S Oliveira, Freni K Tavaria. Novel Strategies for Preventing Dysbiosis in the Oral Cavity. *Front. Biosci.* (Elite Ed) 2023, 15(4), 23. https://doi.org/10.31083/j.fbe1504023
20. Gibson, G., Hutkins, R., Sanders, M. et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol 14,* 491–502 (2017). https://doi.org/10.1038/nrgastro.2017.75
21. Nat Rev Gastroenterol Hepatol. 2014 Aug;11(8):506-14. doi: 10.1038/nrgastro.2014.66. Epub 2014 Jun 10.
22. Goldenberg JZ, Yap C, Lytvyn L, Lo CK, Beardsley J, Mertz D, Johnston BC. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev. 2017 Dec 19;12(12):CD006095. doi: 10.1002/14651858.CD006095.pub4. PMID: 29257353; PMCID: PMC6486212.
23. Cureus. 2024 Feb 25;16(2):e54879. doi: 10.7759/cureus.54879
24. Salminen, S., *et al.* (2021). "Postbiotics—scientific evidence, definition and usage: A consensus statement from the ISAPP." *Nature Reviews Gastroenterology & Hepatology, 18(11), 649-667.*
25. Aguilar-Toalá, J. E., *et al.* (2018). "Postbiotics: An evolving term within the functional foods field." *Trends in Food Science & Technology,* 75, 105-114.
26. Collado, M. C., *et al.* (2019). "Postbiotics: Facts and open questions." *Current Opinion in Biotechnology*, 56, 133-139.
27. Teame, T., *et al.* (2020). "The role of postbiotics in mitigating health risks associated with antibiotic residues in food products." *Frontiers in Microbiology,* 11, 575644.
28. Taverniti, V., & Guglielmetti, S. (2011). "The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept)." Genes & Nutrition, 6(3), 261-274.
29. Shenderov, B. A. (2013). "Gut indigenous microbiota and epigenetics." Microbial Ecology in Health and Disease, 24(1), 20904.
30. Tsilingiri, K., & Rescigno, M. (2013). "Postbiotics: What else?" Frontiers in Microbiology, 4, 58.
31. Rad, A. H. E., *et al.* (2020). "Postbiotics as dynamic biological molecules for improving human health." Medicinal Research Reviews, 40(4), 1336-1375.
32. Atlas of Oral Microbiology, Academic Press, 2015, Pages 41-65, ISBN 9780128022344, https://doi.org/10.1016/B978-0-12-802234-4.00003-(https://www.sciencedirect.com/science/article/pii/B9780128022344000033)
33. Data on file.
34. Data on file.
35. Data on file.



BACKGROUND

Many of the dental diseases have been linked to oral microflora. The oral microbiome contains over 700 bacterial species as well as fungi, viruses, archaea, and protozoa. The alterations increase processes such as the acidification of saliva, the decrease in oxygen which leads to the development of anaerobic bacteria. The oral colonization progress in line with the gut microbiota which constitutes the great biological organ communicating with the other microbiota via cross-talking axes such as oral/lung, gut/lung, gut/brain, gut/skin, gut/liver, and bladder/gut.[1]

The increase in pathogenic bacteria and a corresponding decrease in beneficial bacteria have led to extensive research focused on interventions to restore microbial health and balance in the oral microbiome, including prebiotics, probiotics, and antimicrobial agents, as potential therapeutic approaches for treating and preventing oral dysbiosis and its associated diseases.[2]

Role of Oral Microbiota in Oral Health:[3]

The oral cavity is a warm, moist, and nutrient-rich environment that provides a suitable niche for the colonization of microbiota. Early colonization is exhibited by Gram-positive bacteria, such as Streptococci *(S. sanguinis, S. mutans, S. sobrinus, S. gordonii, and S. oralis)*, as well as Actinomyces, through adhesion to various surfaces, including gingival surfaces and teeth covered by pellicles.

The oral microbiota strives to retain a healthy dynamic state and a symbiotic relationship in the complex oral ecosystem, which is referred to as eubiosis. Positive effects of host-microbiome symbiosis can be seen in (Fig. 1). In addition, these bacteria can self-aggregate and co-aggregate with other bacterial species, including some Gram-negative bacteria, through various bacterial glycoproteins and polysaccharides. This process serves as the basis for biofilm formation. However, this state of eubiosis can be interrupted and transformed into a state of dysbiosis. Dysbiosis is characterized by the involvement of bacterial metabolites in periodontal disease, as well as a marked difference in the metabolic profiles of the oral microbiota when compared with healthy conditions.

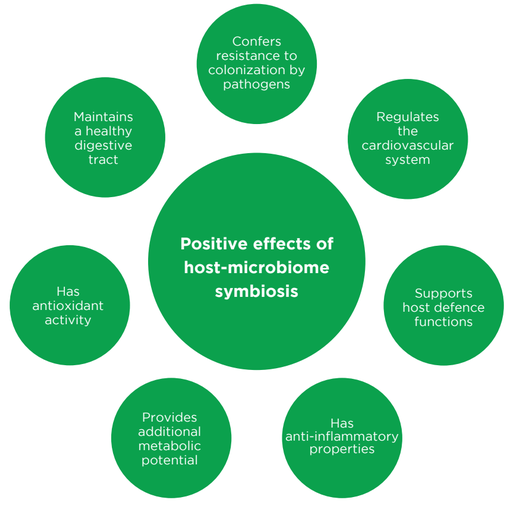


Fig. 1: Positive effects of host-microbiome symbiosis.[4]

Closeed Size: 14.8 Inch x 10.5 Inch

PATHOPHYSIOLOGY OF ORAL DYSBIOSIS

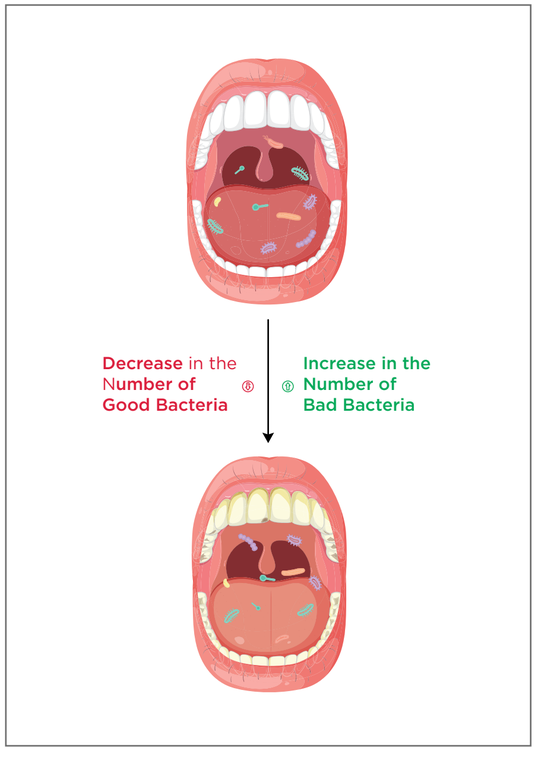
Oral Dysbiosis

An imbalance between the microbiota and the host caused by certain factors can lead to diversity in the microbial community. This can cause oral and dental diseases in which beneficial bacteria are destroyed, and pathogens proliferate. Under these conditions, the immune system is unable to fight the pathogens, exposing the host to tissue and dental damage such as dental caries, periodontal disease.[5]

Dysbiosis:

Oral dysbiosis refers to an imbalance or disruption in this community, which can arise from various factors such as disease, poor oral hygiene, medication use, and diet. Dysbiosis of the oral microbiome can contribute to the development of numerous oral and systemic diseases, including dental caries, periodontitis, oral and other cancers, cardiovascular disease, and diabetes.[6]

Hajishengallis and Lamont proposed a model whereby periodontitis is initiated by a dysbiotic and synergistic microbiota, as opposed to a conventional infectious disease.[5]



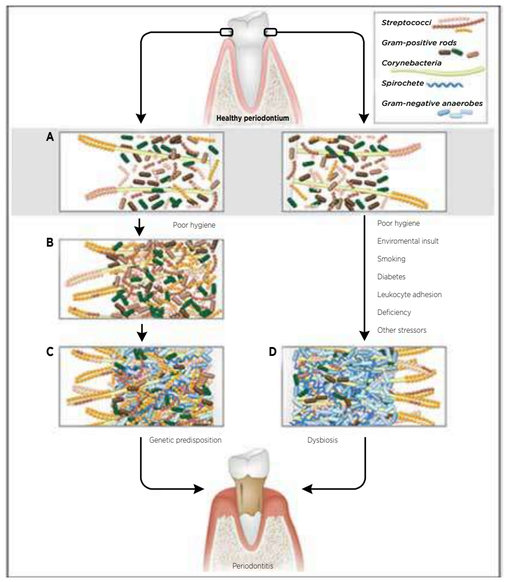


Fig. 2: Alternative models of plaque biofilm development in health and disease.[5]

A. Early biofilm dominated by initial colonizers such as streptococci and Gram-positive rods such as Actinomyces.

B. Mature plaque, with ecological succession that allows for colonization of diverse organisms, including multiple streptococcal and Gram-positive rod species, and later introduction of filamentous forms such as Corynebacteria that serve as scaffolds for streptococci and other species. This stage of development, which results from poor hygiene that allows biofilm development, is associated with gingivitis.

C. Continued succession allowing for colonization by diverse Gram-negative anaerobic species and spirochetes. This stage of development is associated with periodontitis.

D. Aberrant development resulting in dysbiotic plaque biofilm with reduced diversity, as influenced by various environmental perturbations (smoking, diabetes, stress, etc.). Both outcomes require a permissible host genetic background. 1) Streptococci. 2) Gram-positive rods. 3) Corynebacteria. 4) Spirochetes. 5) Gram-negative anaerobe.

PATIENT INFORMATION LEAFLET (FAQS)

- **What is Sporlac-DG?**

  Sporlac-DG is a novel oro-dental tablet that offers heat-killed probiotic based strategy for managing gum diseases, promoting overall oral health, and averting its evolution into advanced gum and dental diseases. It contains *Lactobacillus paracasei* GMNL–33, a type of good bacteria which is beneficial for your dental health.

- **What does Sporlac-DG contain?**
  How does Sporlac-DG work in oral cavity?

  Each tablet of Sporlac-DG contains NLT 1 billion cells of heat-killed *Lactobacillus paracasei* GMNL-33 and 50mg of *Chichorium* intybus root powder.

- **What are heat-killed probiotics?**

  Heat-killed probiotics are described as inactivated probiotics with an intact cell component and structure.

- **What is Sporlac-DG used for?**

  Sporlac-DG corrects the imbalances in oral microbiota. It shifts oral bacterial composition towards a healthier state, suggesting its potential use in various periodontal diseases.

- **How to take Sporlac-DG oro-dental tablet?**

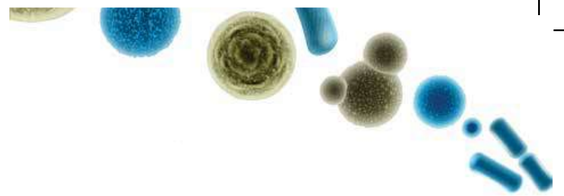
  Place the tablet on the tongue until it dissolves. Do not chew or swallow. Follow the instructions on the pack label.

- **Can Sporlac-DG replace your regular dental care?**

  No, Sporlac-DG is not a replacement for regular dental care like brushing, flossing, and rinsing. It should be used along with your routine dental care to keep your oral cavity infection free and healthy.

- **Who should not take Sporlac-DG?**

  - Those who have known allergies to any components of Sporlac-DG should avoid its use.



  - Individuals with weakened immune systems or serious health conditions should consult a healthcare professional before taking Sporlac-DG to ensure safety.

  - It is advisable for pregnant /lactating women to seek medical advice before using Sporlac-DG.

- **What are the possible side-effects associated with Sporlac-DG?**

  As per the available scientific evidences, no known side effects have been documented.

- **How many tablets of Sporlac-DG can you consume in a day?**

  The general recommendation is to take 1 tablet daily post meal.

- **Is Sporlac-DG gluten or sugar-free?**

  Yes, Sporlac-DG is gluten free and sugar-free.

- **How to store Sporlac-DG oro-dental tablets?**

  Store in a cool and dry place. Keep the tablets out of reach of children.

Closeed Size: 14.8 Inch x 10.5 Inch

5 **Age: Mean age:** 47.7 years

6 **Groups:**
Control group: Placebo (n=20)

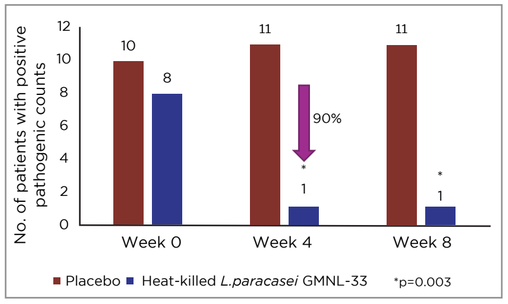
Test group: Heat-killed *L. paracasei* GMNL-33 oral tablet TID (1X10^9 cells) (n=20)

7 **Evaluation parameters:** Periodontal pathogen count

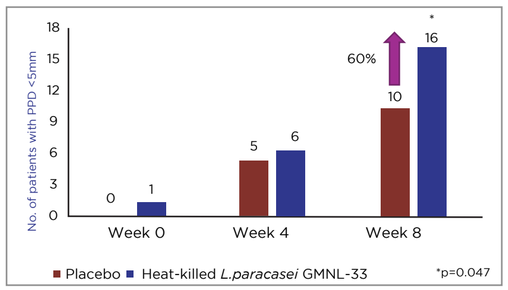
8 **Evaluation time points:** Day 0, week 4 and

Results:

1. Heat-killed *L.paracasei* GMNL-33 showed significant reduction in number of patients with positive periodontal pathogens.



2. Heat-killed *L.paracasei* GMNL-33 showed significant improvement in number of patients with periodontal probing depth <5mm.



Conclusion: Heat-killed *L.paracasei* GMNL-33 tablets significantly improves periodontal health of patients by decreasing count of periodontal pathogens.

Summary

1. Oral diseases, affects nearly 3.5 billion people globally.
2. These diseases are predominantly linked to oral dysbiosis, characterized by an imbalance of pathogenic and beneficial bacteria.
3. To counteract the dysbiosis, management options like antibiotics are being used.
4. However, due to antibiotic resistance and lack of targeted therapy, desired effects are not being achieved.
5. Recently, therapeutic options like prebiotics, probiotics, and heat-killed probiotics are being evaluated.
6. Heat-killed probiotics have following advantages over pre and probiotics:
   - No dependency on live bacteria or existing microbiota.
   - Stable and effective under various conditions (heat, pH)
7. *Lactobacillus paracasei* GMNL-33, a heat-killed probiotic emerges as a promising probiotic in dentistry.
8. *Lactobacillus paracasei* GMNL-33 inhibits harmful bacteria like *Streptococcus Mutans and periodontal pathogens while promoting oral health.*
9. The key features of *Lactobacillus paracasei* GMNL-33 are:
   - Inhibits caries causing bacteria
   - Reduces dental plaque formation
   - Reduces gingival inflammation
10. Clinical studies show it is efficacious and safe across age groups for oral health.

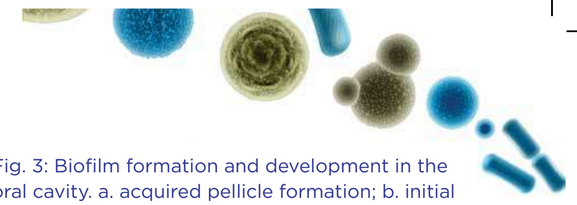


Fig. 3: Biofilm formation and development in the oral cavity. a. acquired pellicle formation; b. initial attachment of early colonizers; c. maturation of biofilm and coaggregation of bacteria; D. dispersion of bacteria.

Biofilm formation and development in the oral cavity:[7]

The specific plaque hypothesis (SPH) emerged which attributed dental caries to specific bacteria in the dental plaque biofilm, mainly *Streptococcus mutans, S. sobrinus, and lactobacilli.*
The shifting of the microbiome from a symbiotic one to a biofilm characterized by dysbiosis induced and aggravated by low abundant 'keystone pathogens'. For example, *P. gingivalis* elicits an intense/destructive host immune response.

Formation of acquired pellicle:

Colonization of organisms within the dental biofilm should be preceded by the presence of a condensed layer of macromolecules at the base of the biofilm known as the acquired pellicle (Fig. 3).

Adhesion of bacteria:

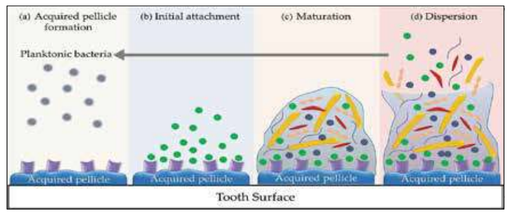
Planktonic bacteria in the oral cavity attach to specific pellicle-associated binding sites such as acidic proline-rich proteins and-amylase for attachment of early colonizers (Fig. 3). In addition to the biofilm matrix, attachment of bacteria within the biofilm is mediated by specialized appendages called fimbriae or pili that are composed of subunits called fimbrillins, possessing adhesins that selectively adhere to pellicle-coated teeth or to other bacteria. Fimbriae are common among many, bacterial species including *Streptococci, Actinomyces and P. gingivalis.*

Maturation of biofilm and coaggregation of bacteria:

Maturation of the dental plaque biofilm (Fig. 3) starts with the recognition by late colonizers including *F. nucleatum, Treponema denticola, T. forsythia, P. gingivalis, P. intermedia,* and *A. actinomycetemcomitans* of polysaccharide or protein-binding sites on the cell surface of primary colonizers.

Dispersion of bacteria:

In general, dispersion of bacteria occurs by three mechanisms: erosion, sloughing, and seeding. While erosion and sloughing may be active or passive, seeding is an active process limited to hollow cavities within the biofilm from which large numbers of solitary cells or bacterial masses are rapidly detached (Fig. 3.)



Factors Influencing Oral Microbiota

The oral microbiome is a complex ecosystem that contribute to oral health. Various factors, such as diet, smoking, alcohol consumption, lifestyle choices, and medical conditions, can affect the balance of the oral microbiome and lead to dysbiosis, which can result in oral health issues like dental caries, gingivitis, periodontitis, oral candidiasis, and halitosis. Novel associations between the oral microbiome and systemic diseases have also been explored in literature including gastrointestinal, cardiovascular, endocrinal, and neurological conditions, autoimmune diseases, and cancer.[8]

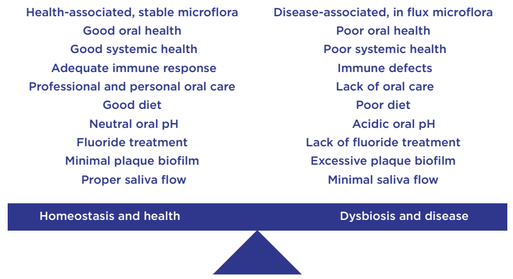


Fig. 4: Illustration of a shift in the homeostatic balance that favors dysbiosis in favor of disease. Influences on the right side that favor disease can include; physical, chemical, or biological influences that can disrupt the balance. Factors such as iatrogenic dentistry, nutrition (excess carbohydrates; type of diet), lack of salivary flow, changes in local pH, defects in enamel mineralization, inadequate immune responsiveness, leukocyte adhesion defects, etc. all can have a profound influence on dental disease at the local level.[9]

Dental plaque is an initiating factor that first causes gingivitis if accumulated, resulting in the loss of collagen locally. Destructive periodontitis will occur gradually if the inflammation is not well controlled, which has been associated with dysbiosis where the diversity, richness, and relative proportions of species in the subgingival microbiota are altered.

Closeed Size: 14.8 Inch x 10.5 Inch

Oral Microbiome and Oral Diseases:[10]

The oral microbiome plays a pivotal role in the development and progression of periodontal diseases.

Dental Caries:

Dental caries driven by bacterial pathogens and characterized by progressive dental hard tissue deterioration. The caries biofilm, a complex and highly active ecosystem, initiates the accumulation of dental plaque on the tooth's surface. Within this environment, various microorganisms colonize, with *S. mitis* and *S. mutans* being among the initial colonizers. The formation of the biofilm begins when a salivary glycoprotein film (dental pellicle) coats a tooth surface.

Gingivitis:

Gingivitis is describe as inflammation of the gums, typically caused by the accumulation of microbial plaque, or bacteria, on the surface of the teeth due to ineffective tooth brushing. Thus, the importance of effective tooth brushing cannot be overstated, as it is essential for removing food debris and preventing the further growth of plaque. Bacteria present in accumulated plaque on the tooth surface can penetrate the gingival tissue, particularly the gingival sulcus, making the marginal area prone to microbial infection.

Common microbial species associated with gingivitis include *P. gingivalis, A. actinomycetemcomitans, Streptococcus sp., Fusobacterium sp., Actinomyces sp., Veillonella sp., Treponema denticola,* and *Prevotella intermedia.* If untreated, gingivitis has the potential to advance to periodontitis, a condition that can lead to irreversible damage not only to the gums but also to the surrounding bone that supports the teeth.

Periodontitis:

Dental plaque bacteria emerge as the principal culprits in the onset of periodontal disease. Plaque exists in both supragingival and subgingival areas, fostering microbial biofilms that thrive on tooth surfaces. The crevicular epithelium and gingival crevice act as pivotal habitats for microbial initiation, supported by gingival crevicular fluid and a favorable anerobic environment.

The red complex bacteria—*P. gingivalis, Treponema denticola,* and *Tannerella forsythia*—emerge as keystone pathogens, triggering an imbalance in host–bacterial interactions and resulting in bone loss. Furthermore, recent investigations highlight the systemic implications of periodontal diseases. Periodontitis not only poses local health risks but also acts as a potential risk factor for systemic conditions like CVD, diabetes, and cancer.

However, *S. mutans*, one of the main pathogens of caries, was negatively correlated with several main pathogens of periodontitis.

Halitosis:[11]

Halitosis, commonly referred to as oral malodor or bad breath, is a frequent oral condition marked by an unpleasing or disagreeable odor that originates within an individual's mouth. Gram-negative bacteria, including *Prevotella* (Bacteroides) *melaninogenica, Treponema denticola, Porphyromonas gingivalis, Porphyromonas endodontalis,* and others like *Enterobacteriaceae, Tannerella forsythia, Eikenella corrodens,* and various *Fusobacterium* species, are implicated as likely contributors to oral malodor.

Clinical Signs and Symptoms of Oral Dysbiosis[12]

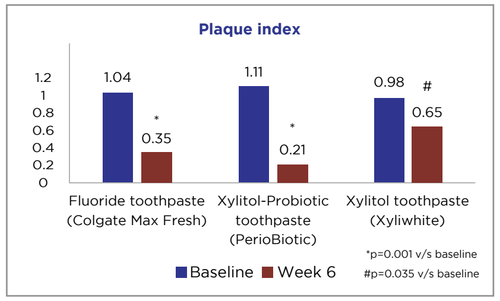
The most common signs include persistent bad breath, bleeding gums, cavities, gum disease (gingivitis and periodontitis), dry mouth, sores and wounds in the mouth, as well as increased tooth sensitivity.

Dental caries (cavities)

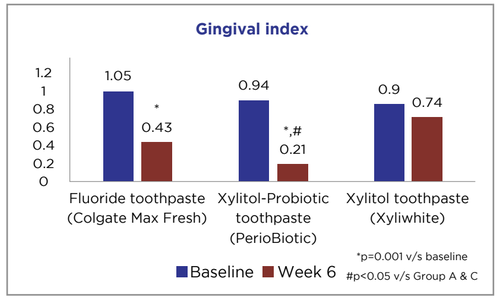
In general, investigation of the oral microbiota composition in caries revealed that the bacterial population in caries is less diverse than in the healthy state. Although, the genera *Streptococcus spp.* and *Lactobacillus* spp., have been studied as the most common causative agents of dental caries, Moreover, *Actinomyces* spp., *Fusobacterium* spp., *Porphyromonas* spp., *Rothia* spp., *Granulicatella* spp., *Gemella* spp., *Selenomonas* spp., *Bifidobacteria* spp., *Scardovia* spp., *Corynebacterium* spp., *Granulicatella* spp., *Propionibacterium* spp., *Bifidobacterium* spp., and *Scardovia* spp. are strongly related to caries development. These microbiotas interact with each other in a dynamic polymicrobial biofilm and induce the progression of caries from early-onset (primary demineralization) to deeper damage with dentin exposure.

Results:

1. Significantly more reduction in plaque index in both fluoride and xylitol-probiotic toothpaste as compared to xylitol toothpaste.



2. Significant decrease in gingival index in xylitol-probiotic group as compared to fluoride and xylitol toothpaste.



Conclusion: Xylitol-Probiotic toothpaste containing heat-killed *L.paracasei* GMNL-33 is an all-round dentrifice that produced a significant reduction in both gingivitis and plaque.

Clinical Study 6:[40]

1. **Aim:** To compare the antimicrobial effect of toothpaste containing fluoride, xylitol or xylitol-probiotic against Streptococcus mutans and Lactobacillus in children
2. **Study design:** Prospective randomized study
3. **Study duration:** 6 weeks
4. **Number of patients:** N = 60 patients
5. **Age:** 13-15 years
6. **Groups:**

   Group A: Fluoride toothpaste

   Group B: Xylitol-probiotic toothpaste (Probiotic –Heat-killed *L.paracasei* GMNL-33)

   Group C: Xylitol toothpaste
7. **Dose:** Brushing twice daily
8. **Evaluation parameters:** *Streptococcus mutans* count in saliva (*S.mutans*)
9. **Evaluation time points:** Pre and post-treatment

Results:

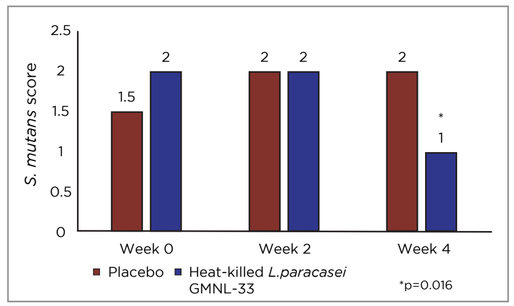
Reduction in salivary *S.mutans* count in xylitol probiotic group is more markedly than fluoride and xylitol toothpaste.

Conclusion: The use of heat-killed *L.paracasei* GMNL-33 ensured the balance between bacterial flora in the oral cavity.

Clinical Study 7:[41]

1. **Aim:** To evaluate the inhibitory effect of orally administered *heat-killed L. paracasei* GMNL-33 on the growth of periodontal pathogens
2. **Study design:** Randomized, double-blind, placebo-controlled study
3. **Study duration:** 8 weeks
4. **Number of patients:** N = 40 patients with periodontitis

Closeed Size: 14.8 Inch x 10.5 Inch



Conclusion: A 2-week period of medication via oral administration route may be needed for heat-killed *L.paracasei* GMNL-33 to be effective in the probiotic action.

Cinical Study 4:[38]

Aim: To analyze the extent of plaque regrowth upon the usage of a probiotic toothpaste containing *L. paracasei* by measuring the optical density using a spectrophotometer

Study design: Pilot study

Age: Mean age of the children was 10.2 years

Number of patients: N = 5 healthy children

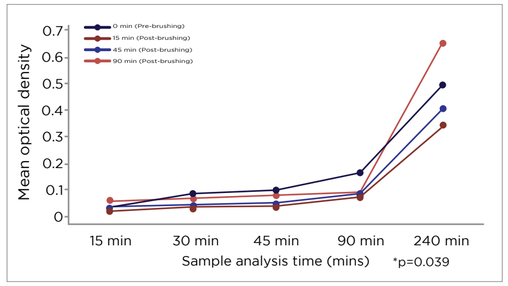
Dose: 0.75gm toothpaste containing heat-killed *L. paracasei* GMNL-33

Evaluation parameters: Optical density of plaque samples

Evaluation time points: 15 min, 45 min, 90 min, and 4 h, respectively

Results:

Plaque regrowth rates



Clinical Study 5:[39]

1. **Aim:** To evaluate the clinical effects of toothpaste containing fluoride, xylitol or xylitol-probiotic on the decrease of dental plaque and gingival inflammation
2. **Study design:** Prospective, randomized, double-blind placebo-controlled clinical trial
3. **Study duration:** 6 weeks
4. **Number of patients:** N = 48 children (adolescents)
5. **Age:** 13-15 years
6. **Groups:**

   Group A: Fluoride toothpaste

   Group B: Xylitol-probiotic toothpaste (Probiotic –Heat-killed *L. paracasei* GMNL-33)

   Group C: Xylitol toothpaste
7. **Dose:** Brushing twice daily
8. **Evaluation parameters:** Gingival index and Plaque index.
9. **Evaluation time points:** Day 0 (Baseline) and Week 6 (Day 42)

Periodontal diseases (gingivitis and periodontitis)

Chronic inflammation caused by oral microbiota can damage the structures that support the teeth, leading to gingivitis and periodontal disease. Gingivitis is a reversible stage that begins at the bottom of the apical gingival border and modifies the supra and subgingival sulci.

EPIDEMIOLOGY AND GLOBAL PREVALENCE OF ORAL DISEASES[13]

The WHO Global Oral Health Status Report (2022) estimated that oral diseases affect close to 3.5 billion people worldwide, with 3 out of 4 people affected living in middle-income countries. Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth.

Periodontal disease affects the tissues that both surround and support the teeth. The disease is characterized by bleeding or swollen gums (gingivitis), pain and sometimes bad breath. In its more severe form, the gum can come away from the tooth and supporting bone, causing teeth to become loose and sometimes fall out. Severe periodontal diseases are estimated to affect around 19% of the global adult population, representing more than 1 billion cases worldwide.

The estimated global average prevalence of complete tooth loss is almost 7% among people aged 20 years or older. For people aged 60 years or older, a much higher global prevalence of 23% has been estimated.

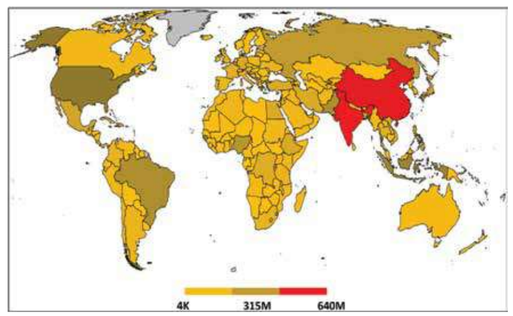


Fig. 5: Country-wise cumulative prevalence of top five leading causes (dental caries [both deciduous and permanent teeth], severe periodontal disease, edentulism and cancer of lip and oral cavity as the leading causes of oral disease burden) of oral diseases (global burden of disease; 2019). India and China had the highest caseloads (632 million and 599 million, respectively) whilst Nauru and Tuvalu had the lowest caseloads (5181 and 4382, respectively). Data accessed from Institute of Health Metrics and Evaluation Global Burden of Disease.[14]

Periodontal diseases, which include gingivitis and periodontitis, are chronic inflammatory diseases, affecting the teeth supporting tissues.[15] Their prevalence is estimated at 45–50% of individuals globally, with 11.2% of individuals diagnosed with severe periodontitis.[15] Periodontal diseases are usually induced by dental plaque (microbial biofilm) and are associated with multiple species of bacteria that increase in numbers due to a dysbiosis of the plaque.[15] Chronic inflammation might promote the formation of periodontal pockets, which alter the redox and nutritional environment and boost the species richness and variety of biofilms leading to dysbiosis.[15] Gingivitis occurs due to the persistent accumulation of microbial biofilms, sometimes exacerbated by local risk factors such as calculus formation, teeth crowding, iatrogenic restoration, all of which are believed to be the main causes for the initiation and progression of gingivitis.[15] If left untreated, gingivitis may then progress into periodontitis: an inflammation characterized by alveolar bone loss, formation of periodontal pockets and ultimately tooth loss. It has been observed that an alteration within the oral and gut microbiome occurs in periodontal diseases patients.[15]

This widespread condition impacts a significant portion of the worldwide population, ranging from 15% to 60%, and is characterized by the emission of an offensive odor from the mouth, nasal passages, and throat regions.[11]

CLINICAL IMPACT OF ORAL DYSBIOSIS ON OVERALL HEALTH[11]

Under healthy conditions, the oral microbiome thrives in a favorable commensal association with its environment, much like other body regions, including the skin, gut, or vagina. However, it is important to acknowledge that under certain circumstances, certain opportunistic microorganisms within the oral microbiome can undergo a shift, becoming harmful pathogens. This transformation can have implications for the development of various oral and systemic diseases. Major oral diseases such as dental caries and periodontal diseases are both caused by and

Closeed Size: 14.8 Inch x 10.5 Inch

contribute to disruptions in the oral microbiota balance, known as dysbiosis. This dysbiosis can extend its effects to various chronic systemic diseases, leading to the initiation or worsening of conditions such as metabolic diseases, CVD, respiratory diseases, rheumatoid arthritis (RA), adverse pregnancy outcomes, IBD, AD, autism spectrum disorders, and oral mucosal diseases.

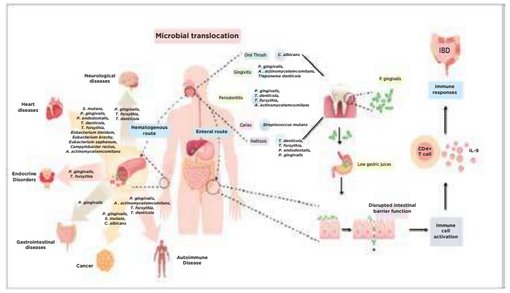


Fig. 6: Oral-Gut pathways to IBD and systemic diseases: Oral microbes such as *Porphyromonas gingivalis* can take two distinct routes to enter the body. The first is the hematogenous route (shown on the left), often associated with dental problems like tooth decay, periodontitis, gingivitis, oral thrush, and halitosis, which can further lead to systemic conditions such as CVD, neurological disorders, autoimmune diseases, diabetes, IBD, and cancer. On the other hand, the enteral route (shown on the right) allows these oral microbes to travel from the stomach to the intestines. *P. gingivalis,* which possesses resistance to antibiotics and can survive stomach acid, moves from the stomach into the gut. Changes in gastric acidity can alter the gut microbiota, making it resemble the oral microbiome. Upon entering the gastrointestinal tract, *P. gingivalis* disrupts the intestinal barrier, compromising gut integrity. This disruption, along with changes in the microbiome, initiates inflammation, typically occurring in the ileum. Within this inflammatory response, specific immune cells known as IL9+ CD4+ lamina propria T cells become active and produce IL-9, a cytokine that fuels immune responses and inflammation. While inflammation serves as a defense against invaders, excessive or chronic inflammation can lead to conditions like IBD. Abbreviations: IL-9: Interleukin-9; IL9+ CD4+ lamina propria T cells: Immune Cells Producing IL-9; IBD: Inflammatory Bowel Disease.[11]

MANAGEMENT OPTIONS

Oral diseases are now considered to be the consequence of a deleterious change in the balance of the oral microbiota, making oral microbiota identification and management a major research axis.

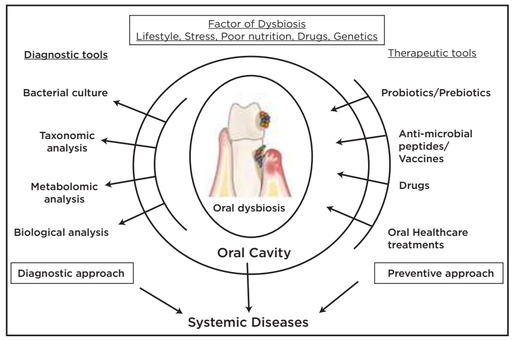


Fig. 7: The oral microbiota is a biomarker, diagnostic tool, and target for systemic disease diagnosis and treatment.[16]

Conventional treatment for oral diseases, the most frequent guideline is to pay regular visits to the dentist, where the focus should be the removal of dental plaque and the implementation of antibiotics where an infection has occurred.[16]

The physical removal of dental plaque, either by brushing the teeth or with professional utensils, such as a scaler, is one of the best traditional strategies to prevent the aggravation of plaque and, consequently, the emergence of caries and gingivitis. However, brushing the teeth is not always effective, and it is not feasible to depend solely on professional actions to prevent oral diseases.[16]

The most common treatment, outside of the dentist's office, to alleviate dental plaque is chlorhexidine mouth rinses. Although its efficacy has been assessed toward different microorganisms, especially *S. mutans,* it has a few disadvantages, such as teeth staining, loss of salivary flow, and the elimination of all microbiota, which eventually worsens the dysbiotic state.[16]

Another important disadvantage in the current treatment of oral caries and periodontitis is the use of antibiotics, particularly broad-spectrum antibiotics. The use of these drugs may not be effective due to the bacterial resistance in the biofilm structure, and their use may also aggravate the resistance to antibiotics while disrupting the oralome and causing a deeper state of dysbiosis.[16]

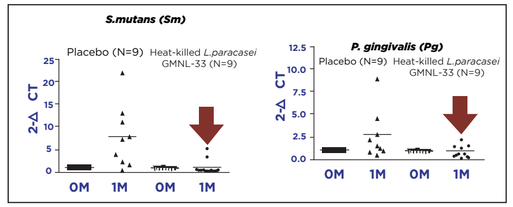
Evaluation parameters:

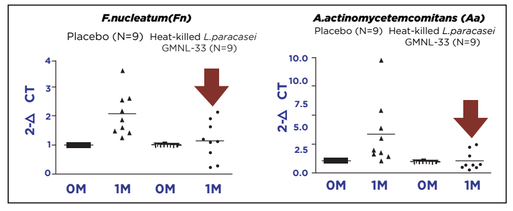
1. Plaque disclosing agent contains a dye that temporarily stains tooth and reveals plaque. Collect dental plaque after gargle
2. Quantification of dental plaque using spectrophotometer
3. Oral bacteria collected by cotton swab and detected by Q-PCR

Evaluation time: Before toothbrush (M0), after 1 month (T1)

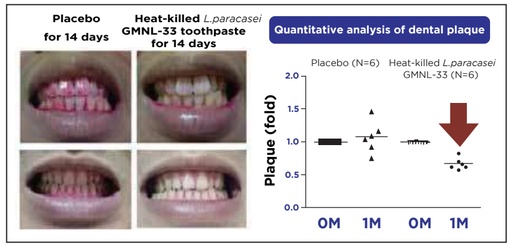
Results:

1. Heat-killed *L.paracasei* GMNL-33 toothpaste decreases the expression of oral pathogens:

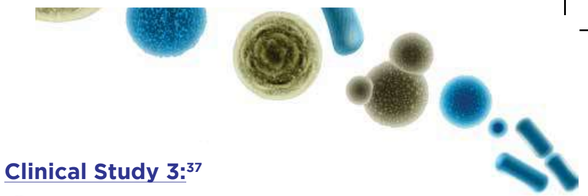




2. Heat-killed *L.paracasei* GMNL-33 toothpaste decreases the dental plaque formation.



Conclusion: Heat-killed *L.paracasei* GMNL-33 toothpaste decreases the oral pathogen count and dental plaque formation.



Clinical Study 3:[37]

1. **Aim:** To evaluate the efficacy of the heat-killed *L.paracasei* GMNL-33 for reducing caries-associated salivary microbial counts in healthy adults
2. **Study design:** Randomized, double-blind, placebo-controlled study with two parallel groups
3. **Study duration:** 2 weeks intervention and 2 weeks post intervention (4 weeks)
4. **Age:** 20-40 years
5. **Number of patients:** N = 78 patients

Group:

1. **Heat-killed Probiotic Group:** 11% xylitol + 4% exact of *L. paracasei* GMNL-33 bacteria 3x10^8 cells/tablet (n=42)
2. **Control Group:** 11% xylitol/tablet (n=36)
3. **Dose:** 1 tablet thrice daily for 2 weeks
4. **Evaluation parameters:** Bacterial counts of salivary *S. mutans*
5. **Evaluation time points:** Baseline (T1), at the completion (T2) of medication and 2 weeks after medication (T3)

Results:

Heat-killed *L.paracasei* GMNL-33 showed significant reduction in the salivary *S. mutants* count.

Closeed Size: 14.8 Inch x 10.5 Inch

PLACE IN THERAPY

1. Gingivitis & Periodontitis
2. Dental Caries
3. Halitosis
4. Decreased Oral Immunity

CLINICAL STUDIES

Clinical Study 1:[35]

Aim: Analysis of the effect of mouthwash containing probiotic heat-killed *L.paracasei* GMNL-33 on oral microbiota

Groups: Control group: Kids Mouthwash 0.01% Heat-killed *L.paracasei* GMNL-33 group: Kids mouthwash+0.01% Heat-killed *L.paracasei* GMNL-33

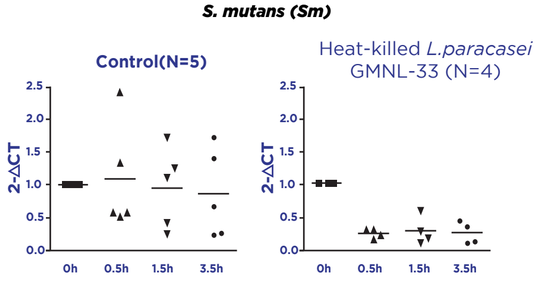
Evaluation parameter: Real time PCR (Cotton swab sampling between gum and teeth)

Evaluation time: Before mouthwash rinsing (T0), after 0.5 hour (T1), after 1.5 hours (T2), and after 3.5 hours (T3)

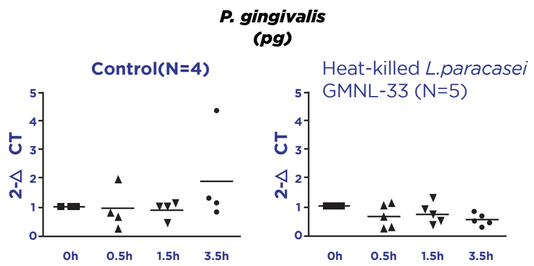
Results:

1. Inhibitory effect of heat-killed *L.paracasei* GMNL-33 mouthwash on oral pathogens:

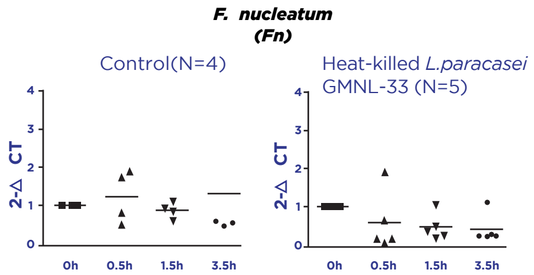
a. *S. mutans*



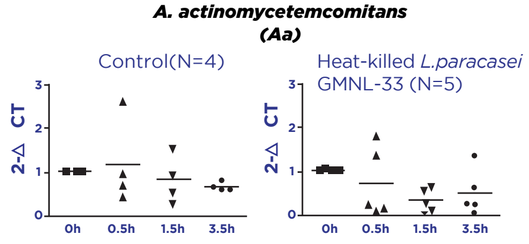
b. *P. gingivalis*



c. *F. nucleatum*



d. *A. actinomycetemcomitans*



Conclusion: Heat-killed *L.paracasei* GMNL-33 inhibits the growth of oral pathogens in children.

Clinical Study 2:[36]

Aim: To evaluate the efficacy of heat-killed *L.paracasei* GMNL-33 in oral health

Groups: Control group: Toothpaste without any anti-bacterial agent
Heat-killed *L.paracasei* GMNL-33 group: Toothpaste containing 0.7% heat-killed *L.paracasei* GMNL-33

Regarding periodontitis, the conventional treatment is based on the physical removal of infra-gingival plaque with a technique called scaling and root planning. Despite being effective in the removal of plaque and lowering the levels of microorganisms in the sulcus, periodontopathogens can re-colonize the subgingival pockets and rapidly enter a dysbiotic state again.[16]

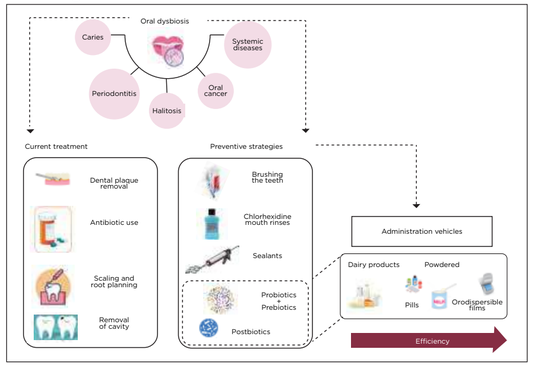


Fig. 8: Oral diseases, current treatments, and preventive strategies.[16]

NEED GAP OF THERAPY

1 Limitations of Conventional Antibiotics in Dentistry:[17-18]

Non-Specific Action: Antibiotics target a broad range of bacteria, including beneficial oral microbiota, leading to dysbiosis and imbalance in the oral microflora.

Antibiotic Resistance: The overuse of antibiotics in dental care contributes to the global rise in antimicrobial resistance (AMR), reducing their long-term effectiveness.

Short-Term Effects: Antibiotics often provide temporary relief from bacterial infections without addressing the root cause, such as biofilm formation or inflammation.

Side Effects: Conventional antibiotics can cause adverse effects such as gastrointestinal disturbances, allergic reactions, or opportunistic infections (e.g., candidiasis).

2 Challenges in Maintaining Balanced Oral Microflora:[19]

Oral Dysbiosis: Factors such as poor oral hygiene, diet, and antibiotic use disrupt the natural balance of oral microbiota, promoting the growth of pathogenic bacteria.

Biofilm Complexity: Dental plaque is a structured biofilm that resists antibiotic penetration, requiring alternative approaches to disrupt its formation.

Recurrent Infections: Conventional treatments often fail to prevent recurrence, as they do not enhance the natural defense mechanisms of the oral cavity.

NOVEL STRATEGIES FOR THE PREVENTION OF ORAL DYSBIOSIS[20]

Oral dysbiosis culminates in oral diseases if the balance is not restored. Interestingly, even though it is one of the most prevalent infectious diseases worldwide, they are still considered a “challenge to modern dentistry.”

One of the most important strategies that must be implemented to prevent oral dysbiosis is controlling biofilm formation. For this reason, novel strategies should focus on the removal of mature biofilms in order to maintain oral homeostasis, especially since it has been proven that oral dysbiosis is the leading cause of the emergence of a pathogenic environment.

Another important factor is that the microorganisms associated with the emergence of oral diseases and oral dysbiosis are mostly endogenous species and not pathogens brought from another environment. Consequently, the treatment and prevention strategies should focus on controlling the growth of these microorganisms rather than their complete removal.

One aspect where current strategies have been failing in the prevention of oral diseases is the maintenance of oral homeostasis or keeping the dysbiotic rate low. This rate is defined as the relationship between the pathobiont species present in the oral environment and the species that are mostly associated with oral health. One approach that should be considered is the use of probiotics, prebiotics, and heat-killed probiotics to control the oral cavity, especially since they can modulate the oral microbiome and influence the dysbiotic rate by controlling the growth of disease-related microorganisms.

Prebiotic is “a substrate that is selectively utilized by host microorganisms conferring a health benefit”.

Probiotic as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”.

Heat-killed probiotics are described as inactivated probiotics with an intact cell components and structure.

Closeed Size: 14.8 Inch x 10.5 Inch

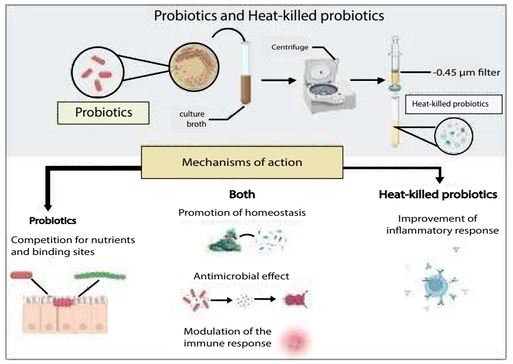


Fig. 9: Mechanisms of action for probiotics and heat-killed probiotics.[20]

Limitations of Prebiotics and Probiotics

1 **Prebiotics**

- **Dependency on Existing Microbiota:** Prebiotics require a healthy microbiota to be effective, limiting their use in dysbiotic conditions.[21]

2 **Prebiotics**

- **Survivability Issues:** Probiotics may not survive gastric acid, bile, or harsh oral conditions, reducing efficacy.[22]
- **Storage Sensitivity:** Probiotics require specific storage conditions for viability.[23]
- **Risk of Infection:** In rare cases, probiotics can cause bacteremia in immunocompromised individuals.[24]

Heat-killed probiotics:

Advantages:

- No dependency on live bacteria or existing microbiota.[25-26]
- Stable and effective under various external and internal conditions (Heat, PH, Enzymes, etc.)

Lactobacillus paracasei GMNL-33 - Role in Oral Health[33]

The *Lactobacillus paracasei* species includes non-motile, non-spore-forming, rod shaped, facultative anaerobic lactic acid bacteria (LAB) that populate several niches, from fermented foodstuffs to host-associated microenvironments. The cell wall of lactobacilli is made up of peptidoglycan, a type of carbohydrate protein complex that helps to give the cell its shape. The cell wall also contains lactic acid, which helps to protect the bacterium from acidic environments. The cytoplasm of lactobacilli is clear and contains a small amount of DNA. In addition to the cell wall and cytoplasm, lactobacilli also have a thin layer of slime that covers their surface. This slime layer helps the bacteria to attach to surfaces and protects them from dehydration. The strain stains Gram-positive.

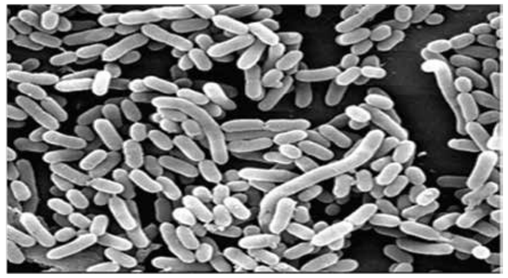
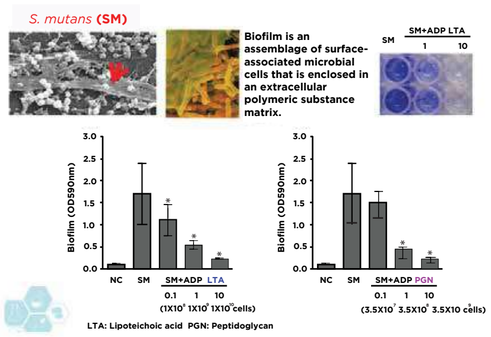


Fig. 10: Lactobacillus casei cell structure[33]

Lactobacillus paracasei GMNL–33, which is effective in reducing dental plaque and gingival formation by inhibiting associated oral pathogens such as *Streptococcus mutans, Porphyromonas gingivalis,* and *Prevotella intermedia.*

Mechanism of action of *L. paracasei* GMNL-33[34]

Lipoteichoic acid and Peptidoglycan are active constituents of *L. paracasei* GMNL-33 present in the cell wall.



1 **Anti-pathogenic activity:**[34]

a. Heat-killed *L.paracasei* GMNL-33 inhibits the periodontal pathogens including *Porphyromonas gingivalis, Prevotella intermedia,Centipeda periodont* and Dental plaque sample collected from teeth with periodontal disease

b. Heat-killed *L.paracasei* GMNL-33 also inhibits growth of dental caries pathogen *S. mutans*

2 **Anti-adhesive:**[35]

Heat-killed *L.paracasei* GMNL-33 inhibits adhesion of *S. mutans* to gingival cavity, also significantly inhibit the biofilm formation of *S. mutans.*

3 **Coaggregration and agglutination:**[36]

Coaggregate to oral pathogens including *S. mutans*, *P. gingivalis, Fusobacterium nucleatum* and *Actinobacillus actinomycetemcomitans* for its removal.

4 **Heat-killed *L.paracasei* GMNL-33 restores oral microflora**

5 **Immunomodulatory activity:**

Heat-killed *L. paracasei* increases salivary IgA levels.

Key characteristics:[42-46]

1 **Targeted Action:**
Heat-killed *L.paracasei* GMNL-33 specifically inhibits caries-causing bacteria (e.g., *Streptococcus mutans*) without harming beneficial microbiota, supporting oral microbial balance.

2 **Biofilm Disruption:**
Heat-killed *L.paracasei* GMNL-33 effectively reduces dental plaque formation by disrupting biofilm integrity, a significant challenge for antibiotics.

3 **Anti-Inflammatory Benefits:**
Heat-killed *L.paracasei* GMNL-33 reduces gingival inflammation by modulating immune responses, providing dual benefits of antimicrobial and anti-inflammatory effects.

4 **No Risk of Resistance:**

Unlike antibiotics, Heat-killed *L.paracasei* GMNL-33 do not contribute to antimicrobial resistance, making them a sustainable alternative.

5 **Enhanced Oral Health:**

By promoting a balanced oral microbiome, heat-killed *L.paracasei* GMNL-33 help in long-term maintenance of oral health, reducing the likelihood of recurrent infections.

Advantages of *L.paracasei* GMNL-33:[42-46]

While antibiotics are reactive, heat-killed probiotics like *L. paracasei* GMNL-33 focus on preventing infections by strengthening the oral ecosystem.

1 **Safety Profile:** Heat-killed *L.paracasei* GMNL-33 are naturally derived and exhibit excellent safety and tolerability, even with prolonged use.

2 **Holistic Benefits:** In addition to addressing oral infections, heat-killed *L.paracasei* GMNL-33 contribute to overall systemic health through their positive effects on inflammation and immunity.

In short, heat-killed probiotic like *L. paracasei* GMNL-33 fills a critical need gap in dentistry by offering a targeted, sustainable, and safe approach to managing oral infections and maintaining microbial balance, areas where conventional antibiotics fall short. Its potential as a non-invasive, adjunctive therapy can revolutionize dental care by reducing reliance on antibiotics and improving long-term oral health outcomes.

Key Features of *L.paracasei* GMNL-33

1 Inhibits caries-causing bacteria *streptococcus* mutans, periodontal pathogens *(Porphyromonas gingivalis, Prevotella intermedia* and *Actinobacillus actinomycetemcomitans)*

2 Reduces dental plaque formation

3 Reduces gingival inflammation

Closeed Size: 14.8 Inch x 10.5 Inch