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Short Communication

Probiotics to reduce microbiota-related dental stains: A potential approach

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Abstract Adult extrinsic black stains on teeth, caused by bacterial colonization, impact aesthetics and confidence. Conventional treatments can be abrasive and have a high recurrence rate. This pilot case study explores probiotics as an adjunctive approach. Direct application of probiotic powder over the black stains of the teeth was carried out prior to routine home care. Results showed black stain removal was possible with tooth brush and dental floss. Saliva and biofilm samples were analyzed via 16S rRNA sequencing. Microbiome revealed a noticeable reduction in *Corynebacterium*, a key black stain-associated bacterium, with slight shifts in major phyla like *Actinobacteriota* and *Firmicutes*. This case study aimed to evaluate the potential of probiotics in reducing black stains on teeth and assess the associated microbiome changes.

Introduction

Extrinsic black stains on adult dentition result from pigment deposition by chromogenic bacteria.[1] Clinically, they appear as irregular black spots or lines along the gingival margin. While not an immediate oral health risk,[1] they notably affect aesthetics, self-esteem, and social interactions. Current management relies on professional scaling and debridement, but recurrence is frequent, and effective long-term treatments remain unavailable.

With recent advancements in oral microbiome research, probiotic therapy has emerged as a potential strategy. By modulating the host microbiota, specific probiotic strains may compete with pathogens, inhibit their proliferation,

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and improve the oral environment. Probiotics can also regulate oral pH and modulate immune responses.[2] While widely used for gastrointestinal health, their role in oral care, particularly against black stains, remains underexplored. Although some studies have examined probiotics in the context of black strains and associated bacteria,[3] most have been in-vitro studies or used to test probiotics as preventive agents.

This case study aimed to evaluate the potential of probiotics in reducing black stains on teeth and assess associated microbiome changes.

Materials and methods

Case description and probiotic application

A 41-year-old systemically and orally healthy male presented with recurrent black dental stains. Despite daily oral hygiene, the stains were generalized, with the most significant accumulation on the maxillary right lateral incisor (Fig. 1A). A commercially available probiotic powder (Chang Ching Pao, 4715872272115, Kaohsiung, Taiwan), containing the strains *Lactobacillus. helveticus LA27*, *L. pentosus LPS16*, *L. plantarum K68*, *L. plantarum LP23*, and *L. rhamnosus* was used to treat the black dental stains (Fig. 1B).

After probiotic application, the recurrence of black stains diminished, as assessed through visual examination during follow-up visits. The presence and extent of black stains were compared before (PreTx) and one week after (PostTx) probiotic use. Although no quantitative tools were employed, a reduced frequency of black stain appearance was noted based on patient feedback and clinical observations.

Tooth surface sampling and clinical data collection

Before treatment (PreTx) and one week after probiotic application (PostTx), swabs were used to collect saliva and biofilms from various intraoral sites, including the labial surfaces of all teeth. To ensure consistency, all collections were performed by the same operator. After collection, the swabs were placed in a sterile storage tube containing 1 mL of TE solution, with the swab collection device broken off and retained inside to seal the tube. Samples were kept on ice until transported to the laboratory.

Next-generation sequencing

Bacterial DNA was extracted using the QIAamp DNA Microbiome Kit (QIAGEN, Hilden, Germany) and sent to Genomics, BioSci & Tech Co. (New Taipei city, Taiwan) for PCR and full-length 16S rRNA sequencing. PCR used primers 27F/1492R with KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington MA, USA). Verified amplicons were pooled, purified with AMPure PB beads (PacBio, Menlo Park, CA, USA), and processed into a SMRTbell library using the SMRTbell Prep Kit 3.0 (PacBio). Sequencing was performed on a PacBio Sequel IIe system, with analysis via the SMRT pipeline v11.0.0.

Statistical analysis

Statistical analyses included Wilcoxon signed rank test to evaluate differences in microbial relative abundance between PreTx and PostTx samples.

Results

Changes in clinical presentation

Before probiotic application, the subject exhibited recurrent black stains, most notably on the upper right central incisor (Fig. 1A). Probiotic powder was applied directly to the stained areas. Followed by regular oral hygiene (toothbrushing and flossing) to aid removal (Fig. 1B). After treatment, visible stain reduction was observed (Fig. 1C), and the subject also self-reported slower stain recurrence.

The sequence data

The biofilm samples were submitted for sequencing. 162,279 gene sequences were collected and 122,664 sequences were qualified after quality control. We identified 4 different phyla, 6 classes, 13 orders, 18 families, 24 genera, and 53 species.

Figure 1 Attenuation of recurrent dental black stains with probiotics, before (A), during (B), and after (C) direct application of the probiotics powder. The stain can be easily removed with toothbrush or dental floss after application. Significant removal of the black stain deposition was noted despite not completely. It is repeatable and the time interval to re-appear the black stain was also prolonged with the intake of probiotics.

Changes in taxa related to tooth black stain

The 4 phyla with greatest relative abundance were *Actinobacteriota*, *Firmicutes*, *Patescibacteria*, and *Proteobacteria* (Fig. 2A). After treatment, *Actinobacteriota* slightly decreased, *Firmicutes* modestly increased, whereas *Patescibacteria* and *Proteobacteria* showed minimal changes.

At the family level (Fig. 2B), *Streptococcaceae* and *Propionibacteriaceae* were the predominant taxa. Specifically, *Streptococcaceae* exhibited a relative abundance exceeding 50%, whereas *Propionibacteriaceae* accounted for approximately 20% in both groups. Lower-abundances families, including *Saccharimonadaceae* and *Burkholderiaceae*, showed comparable relative abundances between the groups, indicating substantial similarity in the overall microbial community composition. Among the top ten abundant families, *Streptococcaceae* showed an increased relative abundance post-treatment, whereas *Corynebacteriaceae* exhibited a notable reduction of approximately 79%. No significant changes were observed in the remaining families.

At the genus level, *Streptococcus* showed an increase in the PostTx sampling, while *Veillonella* decrease (Fig. 2C). *Corynebacterium* exhibited a similar trend in reduction at both genus and species levels (Fig. 2D). Additionally, *Gemella haemolysans* remained consistent.

Discussion

In healthy oral microbiota, *Firmicutes* and *Actinobacteria* are reported as predominant phyla.[4] In our dataset, both groups exhibited *Firmicutes* and *Actinobacteria* as the dominant phyla, with a notable decrease in the relative abundance of *Actinobacteria* in the PostTx sampling. Based on findings by Peterson et al., species associated with dental health include *Capnocytophaga gingivalis*, *Abiotrophia defectiva*, *Lachnospiraceae* spp., *Streptococcus cristatus*, and *Streptococcus sanguinis*.[5] In our samples, *Streptococcus sanguinis* demonstrated a relatively high abundance, with a slight decrease in the PostTx sampling, while *Corynebacterium durum* also declined.

The formation of black stains on the adult dentition is linked to oral microbiota imbalance and influenced by factors such as age, dietary habits, oral hygiene practices, socioeconomic status, and iron supplementation. Bacterial contributors to black stains differ between children and

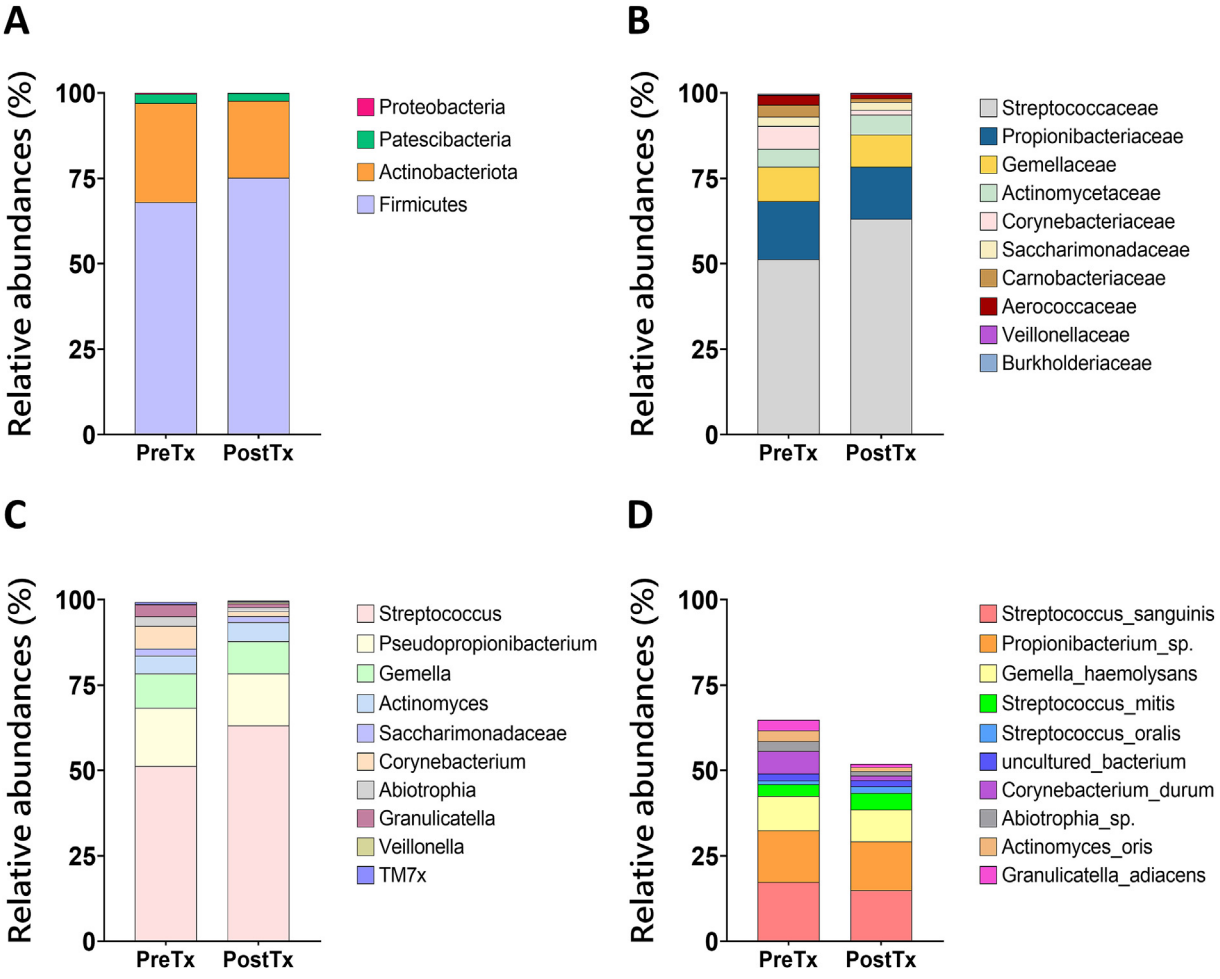

Figure 2 Taxa distribution and differential abundance of black stain microbiota in pre-treatment (PreTx) sampling and post-treatment (PostTx) sampling: stacked prominent phyla (A), families (B), genera (C) and species (D) were presented with mean relative abundance.

adults. In pediatric cases, black stains are commonly associated with *Actinomyces*, *Cardiobacterium*, *Haemophilus*, *Corynebacterium*, *Tannerella* and *Treponema*.[6] In permanent dentition, *Porphyromonas gingivalis*, *Actinomyces* spp., *Aggregatibacter actinomycetemcomitans*, *Streptococci*, *Lactobacilli*, *Prevotella* spp, *Capnocytophaga*, *Corynebacterium*, and *Neisseria* are positively correlated with black stain severity.[7] Current research suggests chromogenic bacteria, notably *Actinomyces*, as primary inducers.[1] Black stains represent a type of bacterial dental plaque containing insoluble iron salts, primarily iron sulfide, formed through bacterial hydrogen sulfide reacting with iron in saliva or gingival crevicular fluid.[8]

In the oral cavity, various probiotic strains have been shown to reduce levels of oral pathogens, inhibit caries formation, and decrease the abundance of bacteria responsible for halitosis.[9] Our preliminary data indicate that probiotics may help mitigate black dental stains by modulating associated microbiota. Clinically, this was associated with an improved esthetic outcome. Probiotics function through competitive exclusion of pathogens, antimicrobial production, and immune modulation. They may inhibit chromogenic bacteria by competing for attachment sites and nutrients or by producing bacteriocins and hydrogen peroxide. *Lactobacillus* and *Bifidobacterium* have demonstrated efficacy in reducing pathogens linked to black stains, caries, and periodontal disease.[9] Despite the availability of probiotic products for oral health, their dosage, efficacy, and safety remain unclear. Currently, no probiotic strain is approved for the treatment, prevention, or cure of oral diseases.

The combined application of probiotics with other therapeutic approaches may be an important area of research. As an adjunct, probiotics may enhance the efficacy of mechanical removal or antibiotics while attenuating black stain recurrence. However, their specific effectiveness, optimal dosage, and application methods require clinical validation. Incorporating probiotics may reduce the need for chemical treatments, lower side effect risks, and provide a more natural, sustainable oral health approach. Future studies should explore their synergy with conventional treatment to maximize therapeutic potential.

Exploring how probiotics modulate the microbiome and immune responses to improve black dental stains is a valuable research direction. Probiotics may suppress immune activation triggered by stain-associated pathogens, reducing local inflammation and promoting a balanced microbial environment. These effects could involve regulating inflammatory mediator expression, influencing dendritic cell and macrophage activity, and modulating cytokine secretion in the oral cavity, thereby supporting anti-inflammatory responses and enhancing oral health.[10] A comprehensive understanding of how probiotics modulate the microbial community and immune responses could provide a scientific foundation for developing targeted probiotic formulations. This knowledge may enable the design of more precise and effective probiotic-based interventions, enhancing their clinical application in personalized oral health management.

The limitations of this study include an insufficient sample size and the absence of controlled trials, limiting the generalizability of the findings. Future research should incorporate larger randomized controlled trials with long-term follow-up to assess the efficacy and sustainability of probiotic therapy for black dental stains. Additionally, evaluating its effects across different age groups and oral health conditions is necessary to determine broader applicability. For patients with coexisting oral diseases, the impact of probiotics may vary, requiring targeted studies on efficacy and safety. In chronic oral conditions, investigating whether probiotics can sustainably reduce black stains and stabilize the microbiota would be valuable. Furthermore, optimizing probiotic dosages and strain combinations is essential to enhance therapeutic outcomes.

In conclusion, this pilot case study supports the potential of using probiotics as an adjunct for reducing black stains. Future research should explore their mechanisms, effects of acidic application, and optimal carriers to enhance therapeutic potential and clinical application.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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