



EFFECT OF BIOGAIA® PRODENTIS IN THE MANAGEMENT OF BLACK STAINS IN CHILDREN: A COMPARATIVE STUDY

Kristina Tabutova, Maria Shindova

Department of Pediatric Dentistry, Faculty of Dental Medicine, Medical University – Plovdiv, Bulgaria.

ABSTRACT:

Aim: To evaluate the clinical effect of the probiotic BioGaia® ProDentis as an adjunctive therapy for the removal of black stains on tooth surfaces in children.

Methods and materials: The study included sixty 5-12-year-old patients with extrinsic black stains, meeting equal inclusion and exclusion criteria. The Modified Lobene Index was used to evaluate the disturbance of black stain pigmentation. Patients were divided into two equal treatment groups. In the experimental group, black stains were removed through professional oral hygiene, followed by the prescription of the probiotic supplement BioGaia® ProDentis for a month, whereas in the control group, only professional oral hygiene was performed. Data processing and analysis were carried out using the statistical software IBM SPSS v 25 (SPSS Inc., IL, USA). An independent sample t-test was used to compare mean differences between two groups.

Results: At the first visit, no significant difference in the distribution of black stain pigmentation was observed between the two groups ($p > 0.05$). By the second visit, the experimental group showed a significant reduction of black stain pigmentation on vestibular and oral surfaces ($p < 0.001$). By the third visit, these beneficial effects remained relatively stable ($p < 0.001$).

Conclusions: The adjunctive use of probiotic BioGaia® ProDentis leads to a progressive reduction of black tooth stains in pediatric patients, achieving statistical and clinical significance at the final assessment.

Keywords: black stain, probiotics, *Lactobacillus reuteri*

INTRODUCTION

Extrinsic black stains appear as dark dots or a continuous line of pigmentation, typically located along the cervical third of the tooth and can be spread on the oral and vestibular surfaces of both primary and permanent dentition [1, 2, 3].

Its etiology has been widely debated for more than a century, with most investigations focusing on pediatric patients [4, 5]. Reports published worldwide indicate a prevalence ranging from 2% to 20%, with no significant differences between genders [6, 7]. Recent research has highlighted a relationship between the presence of black stains and dental caries, suggesting a protective effect [8, 9, 10]. The dark color of the deposits has long been hypothesized to originate from metallic compounds (iron or copper salts) that react with sulfide ions, mainly hydrogen sulfide produced by oral bacteria. This leads to the formation of ferric sulfide, which gives its characteristic black appearance [2, 10]. Previous studies have identified specific microbial species associated with the formation of black stains, revealing distinct differences from conventional dental biofilm and the predominance of specific microorganisms [9, 10].

Oral probiotics, particularly *Lactobacillus reuteri* and *S. salivarius*, have shown potential as promising agents capable of modulating the oral microbiome by suppressing pathogenic species, producing antimicrobial metabolites such as reuterin, and influencing host immune responses. Probiotics can reduce plaque accumulation and gingival inflammation. Clinical evidence supports their potential as a safe and effective product for maintaining oral health in children [11, 12].

Considering the challenge of managing extrinsic black stains in children, this study aims to evaluate the effectiveness of probiotic treatment in reducing the intensity of black stains, compared to conventional professional oral hygiene procedures.

MATERIAL AND METHODS

The study was conducted on sixty 5-12-year-old children (mean age = 8.57 ± 1.75 years). A convenience sample of children was randomly selected from patients who met the study's inclusion and exclusion criteria and were treated at the Department of Pediatric Dentistry in

Plovdiv, Bulgaria, during the period January-June 2024.

The inclusion criteria for patient selection were:

- Age: 5-12 years (mixed or permanent dentition)
- Signed informed consent from the parents for participation in the study
- Presence of black stains on the tooth surface affecting at least six teeth
- Children without a history of allergy to the applied products

The exclusion criteria for patient selection were:

- Medically compromised children
- Children with acute inflammatory processes in the examined area
- Children with multiple carious lesions
- Patients with exogenous pigmentations on the tooth surfaces of other colors
- Patients taking anti-inflammatory drugs, antibiotics or other medications
- Patients receiving homeopathic remedies
- Patients whose dietary habits include regular consumption of seafood, coffee, caffeinated drinks, tea, soy sauce or curry.

The study protocol has been approved by the Committee for Scientific Research Ethics at the Medical University of Plovdiv, Bulgaria (protocol of approval No. 6/05.10.2023).

The clinical assessment of the black stain distribution on tooth surfaces was performed using the Modified Lobene Index (MLI) (Lobene RR 1989, Macpherson 2000), which evaluates both intensity and extent of the stains. This modified index demonstrates precision by taking into account the localization, intensity, and distribution of the stain. The index was applied to the maxillary and mandibular central and lateral incisors, with the corresponding teeth examined according to the child's dentition stage: permanent incisors (12, 11, 21, 22, 31, 32, 41, 42) in children with permanent dentition, and deciduous incisors (52, 51, 61, 62, 72, 71, 81, 82) in children with primary or mixed dentition. Both vestibular and oral surfaces are assessed across four zones: gingival (G), mesial (M), distal (D), and body of the tooth (B). Intensity is coded from 0 (no stain) to 3 (dark brown/black), while extent is coded based on surface coverage (1–3) and only evaluated if intensity is ≥ 2 . The abbreviations include I (intensity), A (extent), IxA (GBMD) and IxA (GB). Intensity and extent are evaluated across all four zones of eight teeth (32 zones total), and IxA (GB) using only the gingival and body areas (16 zones). Average scores are calculated for each patient [13].

The study was a controlled trial with the sample divided into two groups randomly: group I (experimental group) received a professional oral hygiene session and oral hygiene education and motivation, followed by a therapy with a probiotic food supplement containing *L. reuteri* (BioGaia® ProDentis) twice daily for a duration of 1 month. Group II (control group) received only a professional oral hygiene procedure, oral hygiene education and motivation.

The professional oral hygiene procedure included careful removal of black stain deposits and dental plaque

using a Prophy-Mate Neo® air-polishing device (NSK, Japan) with Soft Pearl® glycine polishing powder (NSK, Japan). Glycine air-polishing is recognized as a low-abrasive, well-tolerated method for biofilm and extrinsic stain removal, particularly suitable for pediatric use [14]. The use of air-flow technology provided thorough cleaning of the vestibular and oral surfaces while preserving the integrity of the enamel. This standardized protocol ensured that both groups started the study with comparable oral hygiene status. The patients were allocated randomly to group I or II. All patients were examined by the same operator. Their parents were informed of the purpose and methods of the study, and written consent to participation was then obtained.

The operative methodology of the clinical study followed a diagnostic-therapeutic protocol structured in three visits. The effectiveness of the management approaches was evaluated at the 1st, 3rd, and 6th months.

At the first visit, patients were screened for eligibility and enrolled in the study. Clinical examination included photographic documentation and assessment of black stains using the Modified Lobene index. A professional oral hygiene session was performed, and patients received instructions for home care. Participants of the experimental group were prescribed a probiotic and instructed on its use. Patients were instructed to let the tablet dissolve in the mouth after morning and evening personal oral hygiene (twice daily). The prescribed probiotic supplement was BioGaia® ProDentis (Stockholm, Sweden), provided as lozenges containing *Lactobacillus reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289. Each tablet delivers at least 200 million live cells of *L. reuteri*. Patients were advised not to take any additional probiotics or antiseptic products during the study.

At the second visit, 3 months after visit 1, photographic documentation was repeated, oral hygiene was reevaluated, and the presence of black stains was recorded through the Modified Lobene index, and all patients were re-motivated for personal oral hygiene.

At the third visit, 6 months after visit 1, the last check-up included photographic documentation and a record of the modified Lobene index.

The data collected across the three visits were analyzed using IBM SPSS v 25 (SPSS Inc., IL, USA). Mean values for each parameter were calculated for both groups to allow comparisons. Statistical comparisons of the indices between groups were performed using independent samples t-tests. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Before the start of the therapeutic approach, the four investigated parameters vary as follows:

- For vestibular surfaces - vestibular intensity (Vestibular I) from 25.63 ± 18.7 to 31.30 ± 17.18 , vestibular extent (Vestibular A) from 19.97 ± 12.26 to 23.60 ± 11.10 , vestibular surfaces: gingival, body, distal and mesial (Vestibular IxA GBDM) from 36.80 ± 37.31 to 43.63 ± 30.84 and vestibular surfaces: gingival and body (Vestibular IxA GB) from 10.97 ± 12.93 to 15.83 ± 13.90

- For oral surfaces - Oral intensity (Oral I) from 42.80 ± 18.30 to 47.17 ± 12.41 , Oral extent (Oral A) from 31.63 ± 11.48 to 32.50 ± 9.16 , oral surfaces: gingival, body, distal and mesial (Oral IxA GBDM) from 66.57 ± 42.05 to 63.67 ± 31.51 and oral surfaces: gingival and body (Oral IxA GB)

from 23.93 ± 17.50 to 25.97 ± 17.37 .

Independent sample t-test analysis showed no statistically significant difference in the initial values of the four investigated vestibular and oral parameters of the Modified Lobene Index between the two groups ($p > 0.05$) (table 1, table 2).

Table 1. Mean values (Mean $\pm$ SD) of vestibular parameters for the experimental and control groups in relation to black stain pigmentation over the three visits.

Surface	Visit	Experimental group /N=30/	Control group /N=30/	p-value
Vestibular I	Visit 1	25.63 ± 18.7	31.30 ± 17.18	0.227
	Visit 2	11.77 ± 13.79	25.67 ± 14.94	$< 0.001^*$
	Visit 3	13.33 ± 14.53	29.27 ± 15.95	$< 0.001^*$
Vestibular A	Visit 1	19.97 ± 12.26	23.60 ± 11.10	0.234
	Visit 2	9.43 ± 9.55	20.07 ± 10.25	$< 0.001^*$
	Visit 3	11.10 ± 10.53	22.63 ± 10.56	$< 0.001^*$
Vestibular IxA (GBDM)	Visit 1	36.80 ± 37.31	43.63 ± 30.84	0.443
	Visit 2	13.97 ± 19.65	29.90 ± 22.54	$= 0.005^*$
	Visit 3	16.27 ± 21.45	38.17 ± 26.74	$= 0.001^*$
Vestibular IxA (GB)	Visit 1	10.97 ± 12.93	15.83 ± 13.90	0.166
	Visit 2	2.43 ± 3.78	9.60 ± 9.79	$< 0.001^*$
	Visit 3	2.90 ± 4.62	12.67 ± 11.45	$< 0.001^*$

*statistically significant difference, $p < 0.05$

Table 2. Mean values (Mean $\pm$ SD) of oral parameters for the experimental and control groups in relation to black stain pigmentation over the three visits.

Surface	Visit	Experimental group /N=30/	Control group /N=30/	p-value
Oral I	Visit 1	42.80 ± 18.30	47.17 ± 12.41	0.284
	Visit 2	20.77 ± 10.84	38.63 ± 11.86	$< 0.001^*$
	Visit 3	22.57 ± 10.73	43.90 ± 12.02	$< 0.001^*$
Oral A	Visit 1	31.63 ± 11.48	32.50 ± 9.16	0.748
	Visit 2	17.27 ± 7.12	28.50 ± 8.40	$< 0.001^*$
	Visit 3	19.27 ± 7.11	30.97 ± 8.66	$< 0.001^*$
Oral IxA (GBDM)	Visit 1	66.57 ± 42.05	63.67 ± 31.51	0.763
	Visit 2	23.80 ± 16.43	46.23 ± 24.59	$< 0.001^*$
	Visit 3	26.37 ± 17.99	55.63 ± 27.85	$< 0.001^*$
Oral IxA (GB)	Visit 1	23.93 ± 17.50	25.97 ± 17.37	0.653
	Visit 2	6.93 ± 5.21	17.23 ± 12.43	$< 0.001^*$
	Visit 3	7.87 ± 5.48	20.90 ± 13.11	$< 0.001^*$

*statistically significant difference, $p < 0.05$

After 3 months (during the second visit), the results of our study show that within both groups, there was a reduction of the black stain pigmentation on vestibular

and oral surfaces (Table 1 and Table 2). When comparing the studied parameters of the Modified Lobene Index between the two treatment groups during the second visit,

we found differences that vary significantly. A statistically significant reduction was found across all measured variables in the experimental group, demonstrating the effectiveness of the oral probiotics in reducing vestibular and oral distribution of extrinsic black stain over time compared with the control group ($p < 0.001$).

After 6 months (during the third dental visit), a recurrence of the black stain pigmentation was detected in both groups. However, the study results again reported a significant difference in the values investigated of the Modified Lobene Index between the two groups ($p < 0.001$).

Tables 1 and 2, as well as Figures 1 and 2 in this study, visualize the research trend based on the parameters investigated.

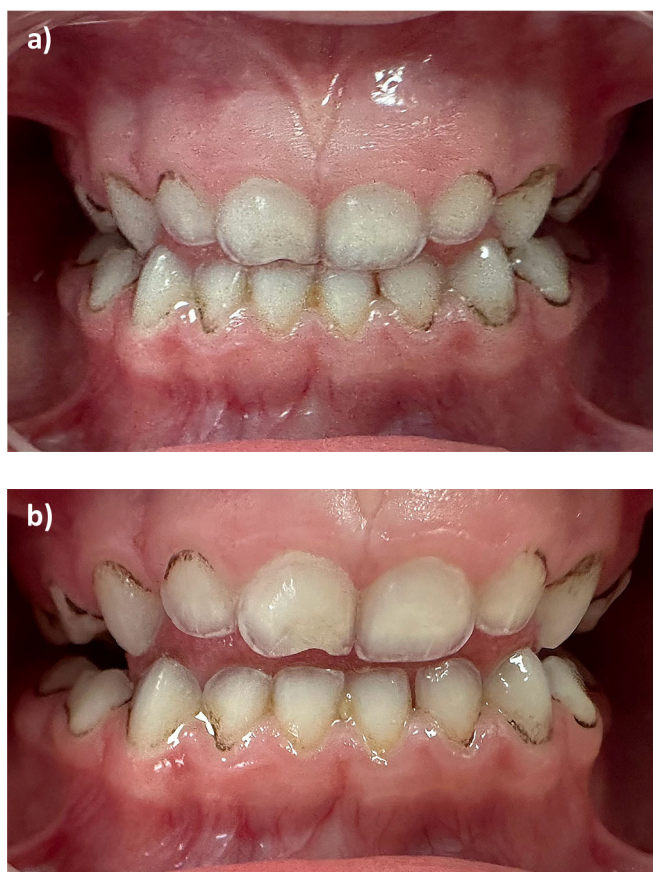
Fig. 1. Clinical aspect after probiotic therapy during a 6-month follow-up period.

A) A 5-year-old boy presents with linear black stains on deciduous dentition. At the first visit (before professional oral hygiene), black stain deposits are visible along the cervical third of both maxillary and mandibular anterior and posterior teeth, predominantly distributed on the oral and vestibular surfaces. Management included mechanical removal with an air-flow device, followed by prescription of the probiotic BioGaia® ProDentis. **B)** At the second follow-up (3 months after the initial check-up), black stains showed a significant reduction in both intensity and extension compared to the first visit. **C)** At the third follow-up (6 months after the second visit), the clinical presentation demonstrated a substantial improvement compared with the initial examination.



Fig. 2. Clinical aspect after professional oral hygiene procedure during a 6-month follow-up period.

A) A 5-year-old girl presented with black stains on mixed dentition. At the first visit (prior to professional oral hygiene), management included mechanical removal of the deposits with an air-flow device. **B)** After 3 months, recurrence of linear and punctiform pigmentations was observed, mainly around the gingival area. **C)** At the third follow-up (6 months), the clinical presentation remained unchanged compared to the 3-month examination, indicating no further improvement.





The findings indicate that both applied methods demonstrated efficacy in reducing the intensity and extent of the black stains, confirming their potential as effective therapeutic strategies. However, the magnitude of the effect was not identical. The first method produced a more pronounced and consistent reduction compared to the second, suggesting superior effectiveness under the given experimental conditions. This difference may be attributed to variations in the mechanisms of action, intensity of microbial inhibition or the sustainability of the achieved results. Nevertheless, the overall data support the view that both approaches are beneficial, while the first offers a greater therapeutic impact.

DISCUSSION

The International Scientific Association for Probiotics and Prebiotics (ISAPP) defines probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. In 2002, the joint commission of the Food and Agriculture Organization-World Health Organization (FAO-WHO) defined probiotics as “live microorganisms, mainly bacteria, safe for humans, which when administered in adequate quantities confer a beneficial effect on the health of the host”. Currently, several microbial species are recognized as safe for human use, including *Lactobacillus reuteri*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Saccharomyces cerevisiae*, and *Carnobacterium maltaromaticum* [15].

L. reuteri is one of the most extensively studied probiotic strains in dental medicine. Probiotics act mainly by regulating microbiota, modulating immune responses, and influencing metabolism. For probiotic strains to exert beneficial effects in the oral cavity, they must be able to colonize or at least temporarily harbor it by adhering to both hard and soft tissue surfaces [16].

In dental medicine, probiotics remain a contemporary approach for different conditions, working mainly by creating an unfavorable environment for pathogens and reducing their virulence [15].

The studies on probiotics and extrinsic black stains are still limited. Only a few have investigated this relationship [15, 17, 18, 19].

The first evidence was provided by Bardellini et al. in 2020, who demonstrated the antagonistic activity of *Streptococcus salivarius* M18 (SsM18) against microor-

ganisms associated with black stains. Fifty-eight children, aged 4-10 years, with existing black stains were enrolled and randomly assigned to receive either SsM18 supplementation or no treatment. After 3 months, black stains were present in 21.2% of the SsM18 group compared to 50% in the control group. After 6 months, the figures were 32.1% vs. 53.8%, respectively. Their findings demonstrated a reduction in the prevalence and severity of black stains in children and suggested that supplementation with SsM18 could prevent black stain formation among children [17].

The following year, D’Errico et al. (2021) conducted a pilot randomized trial evaluating a probiotic supplement that contains *L. reuteri* in school-aged children. Twenty participants aged between 8 and 24 years were randomly divided into two groups. The first experimental group (10 patients) received professional oral hygiene and BioGaia® ProDentis for a duration of two months. The second control group (10 patients) received only a professional oral hygiene session. Black stains were assessed using the Modified Lobene Index at every visit. The mean values of the Lobene index in the test group were lower compared to the control group at the 1st and 2nd visits from the beginning of the treatment. Probiotic supplementation significantly reduced dental biofilm and gingival inflammation through modulation of the oral microbiome and antimicrobial effects of compounds such as reuterin [15].

Similarly, Borrell García et al. (2021) conducted a pilot randomized clinical trial evaluating the use of *L. reuteri* DSM 17938 and ATCC PTA 5289 on oral health indexes in school-aged children, reporting improvements in plaque and gingival indices [18].

The latest microbiological in vitro investigation (2025) demonstrated a significant decrease in the viable counts of *A. actinomycetemcomitans* and *A. naeslundii*, two species associated with black stain formation. The reduction was particularly marked for *A. actinomycetemcomitans* at elevated probiotic concentrations. These findings are consistent with previous reports demonstrating inhibitory activity of *Streptococcus salivarius* M18 and *L. reuteri* against extrinsic black stain-associated bacteria [19].

The results of the study contrasted with a recent case report by Yu et al., which observed only minor changes in Actinomyces levels after topical application of a multi-strain Lactobacillus probiotic powder (containing *L. helveticus*, *L. pentosus*, and *L. rhamnosus*) in a healthy adult. The same study reported a 79% reduction in *Corynebacteriaceae* and improvement in extrinsic black stains, but it was unclear whether these effects were attributable to the probiotic strains or to mechanical abrasion from brushing [20].

In conclusion, these findings suggest that specific probiotic strains may offer therapeutic benefits against extrinsic black stains. However, further focused studies are required to clarify their clinical efficacy and long-term impact. These reports, together with our findings, strengthen evidence for the potential role of probiotics in preventing extrinsic black stains.

CONCLUSION

Our findings confirm, consistent with previous research, that black stains tend to recur over time. In conclusion, it was found that supplementation with *L. reuteri* (BioGaia® ProDentis), administered twice daily for one month, significantly reduced both the extent and intensity of black stain pigmentation compared to the profes-

sional oral hygiene procedure alone. Although this intervention does not completely eliminate black stains, it appears to facilitate their management and decrease recurrence. Further investigations are needed to determine relapse rates and establish the optimal duration of probiotic therapy.

REFERENCES:

1. Zerman N. Black stains in children: a new classification and an innovative flowchart for management and recurrence prevention. *Eur J Paediatr Dent*. 2024 Dec;25(4):277–83. [[Crossref](#)]
2. Gasparetto A, Conrado CA, Maciel SM, Miyamoto EY, Chicarelli M, Zanata RL. Prevalence of black tooth stains and dental caries in Brazilian schoolchildren. *Braz Dent J*. 2003;14(3):157–61. [[Crossref](#)]
3. Iyia T, Kawala B, Antoszewska-Smith J, Kawala M. Black stain and dental caries: a review of the literature. *BioMed Res Int*. 2015;2015:469392. [[PubMed](#)]
4. Mathews LM, Ravikumar D. Prevalence of black stains in school going children in age group 6-12 years. *Indian J Public Health Res Dev*. 2020 Jun 26;11(6):1104–8. [[Crossref](#)]
5. Garan A, Akyüz S, Öztürk LK, Yarat A. Salivary parameters and caries indices in children with black tooth stains. *J Clin Pediatr Dent*. 2012; 36(3):285–8. [[PubMed](#)]
6. Janjua U, Bahia G, Barry S. Black staining: an overview for the general dental practitioner. *Br Dent J*. 2022 Jun;232(12):857–60. [[Crossref](#)]
7. Garcia Martin JM, Gonzalez Garcia M, Seoane Leston J, Llorente Pendas S, Diaz Martin JJ, Garcia Pola MJ. Prevalence of black stain and associated risk factors in preschool Spanish children. *Pediatr Int*. 2013 Jun;55(3):355-9. [[Crossref](#)]
8. Mousa HRF, Radwan MZ, Wassif GOM, Wassel MO. The association between black stain and lower risk of dental caries in children: a systematic review and meta-analysis. *J Egypt Public Health Assoc*. 2022 Jul 30;97(1):13. [[Crossref](#)]
9. Qiao C, Han R, Yang J, Huang H, Ma L. Black stain and dental caries in primary dentition of preschool children in Qingdao, China. *J Clin Pediatr Dent*. 2024 Jul;48(4):200–5. [[Crossref](#)]
10. Zheng L, Cao T, Xiong P, Ma Y, Wei L, Wang J. Characterization of the oral microbiome and gut microbiome of dental caries and extrinsic black stain in preschool children. *Front Microbiol*. 2023 Mar 31; 14:1081629. [[PubMed](#)]
11. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the probiotic *Lactobacillus reuteri*. *Swed Dent J*. 2006; 30(2):55–60. [[PubMed](#)]
12. Burton JP, Wescombe PA, Macklaim JM, Chai MHC, MacDonald K, Hale JDF, et al. Persistence of the oral probiotic *Streptococcus salivarius* M18 is dose dependent and megaplasmid transfer can augment their bacteriocin production and adhesion characteristics. *PLoS One*. 2013 Jun 13;8(6):e65991. [[PubMed](#)]
13. Macpherson LMD, Stephen KW, Joiner A, Schäfer F, Huntington E. Comparison of a conventional and modified tooth stain index. *J Clin Periodontol*. 2000 Nov;27(11):854–9. [[PubMed](#)]
14. Petersilka GJ. Subgingival air-polishing in the treatment of periodontal biofilm infections. *Periodontol 2000*. 2011 Feb;55(1): 124-42. [[Crossref](#)]
15. D’Errico G, Bianco E, Maddalone M, Tregambi E. Usage of *Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289 in the treatment of the patient with black stains. *World J Dent*. 2021 Feb 1;12(1):32–7. [[Crossref](#)]
16. Stamatova I, Meurman JH, Kari K, Tervahartiala T, Sorsa T, Baltadjieva M. Safety issues of *Lactobacillus bulgaricus* with respect to human gelatinases in vitro. *FEMS Immunol Med Microbiol*. 2007 Oct;51(1):194–200. [[PubMed](#)]
17. Bardellini E, Amadori F, Gobbi E, Ferri A, Conti G, Majorana A. Does *Streptococcus salivarius* strain M18 assumption make black stains disappear in children? *Oral Health Prev Dent*. 2020 Apr 3;18(2):161–4. [[PubMed](#)]
18. Borrell García C, Ribelles Llop M, García Esparza MÁ, Flichy-Fernández AJ, Marqués Martínez L, Izquierdo Fort R. The use of *Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289 on oral health indexes in a school population: a pilot randomized clinical trial. *Int J Immunopathol Pharmacol*. 2021 Jan-Dec; 35:20587384211031107. [[PubMed](#)]
19. Wahba N, Sharaf D, Wagdy M, El-Motayam A, Nagi B. The potential bactericidal effect of oral probiotics on extrinsic black stain-associated bacteria in children: an in-vitro and in-vivo study. *J Dent Sci*. 2025 Oct; 20(4):2315-2321. [[PubMed](#)]
20. Yu JJ, Hernandez-Kapila Y, Wang CW. Probiotics to reduce microbiota-related dental stains: a potential approach. *J Dent Sci*. 2025 Jul;20(3):1890-1893. [[Crossref](#)]

Please cite this article as: Tabutova, K Shindova M. Effect of BioGaia® ProDentis in the management of black stains in children: a comparative study. *J of IMAB*. 2026 Apr-Jun;32(2):6913-6919.
[Crossref - <https://doi.org/10.5272/jimab.2026322.6913>]

Received: 01/10/2025; Published online: 11/06/2026



Address for correspondence:

Kristina Tabutova, DMD, Assistant Professor,
Department of Pediatric Dentistry, Faculty of Dental Medicine, Medical University – Plovdiv
3, Hristo Botev Blvd., 4000 Plovdiv, Bulgaria.
E-mail: kristinatabutova7@gmail.com,