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# Usage of *Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289 in the Treatment of the Patient with Black Stains

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## ABSTRACT

**Aim:** This pilot study aimed to evaluate the effectiveness of an oral probiotic, *Lactobacillus reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 (ProDentis®, BioGaia), to avoid the recurrence of black stains (BS) in children and young adults. The purpose of this work is also to propose an oral hygiene protocol that could hinder or slow down the formation of these pigments.

**Materials and methods:** Twenty patients aged between 8 years and 24 years with extrinsic pigmentations on one or more dental elements and with black lines attributable to chromogenic bacteria were divided into two groups randomly: the first (group I with 10 patients, test group) received a professional oral hygiene session and a therapy with a probiotic food supply containing *L. reuteri* for a duration of 2 months. The second group (group II with 10 patients, control group) received only a professional oral hygiene session. Data were collected using the Lobene modified index.

**Results:** The mean Lobene index is lower in the test group compared to the control group after both 1 and 2 months from the beginning of the treatment.

**Conclusion:** Black stain formation could be prevented by administering an *L. reuteri*-based probiotic food supply.

**Clinical significance:** The use of probiotics is a relatively undeveloped strategy for the prevention and treatment of BS.

**Keywords:** Black stain, Caries prevention, Lactobacillus, Occlusion, Periodontitis, Probiotic.

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## INTRODUCTION

Black stains (BS) are characterized by a black line or an incomplete coalescence of dark spots localized on the cervical third of the tooth. Over the past century, the etiology of these pigmentations had been debated. Most of the studies on this problem were conducted on the pediatric population.<sup>1,2</sup>

Dental pigmentations often represent an esthetic problem and are classified as extrinsic and intrinsic according to their location on the tooth. Extrinsic pigmentations originate in the acquired dental film and are exclusively localized on the tooth surface. These spots are usually associated with the presence of bacterial colonies, poor oral hygiene, the use of tobacco and its derivatives, the intake of highly coloring foods and drinks, the use of chlorhexidine or other drugs.<sup>2</sup> Intrinsic pigmentations instead involve deeply the hard tissues of the tooth and can be primitive, if they manifest themselves on enamel and dentine during their formation, or acquired dental dyschromias, determined by external causes, such as, aging, amalgam restorations, trauma, pulp necrosis, or endodontic treatments.

Black stains are a particular type of extrinsic pigmentation. They were described by Wilkins in 2005 as black spots having a linear conformation and showing incomplete coalescence points, characteristic of this pigmentation, which rarely extend beyond the third cervical crown, but which can also affect the base of tooth ridges and dental pits.

In literature, the prevalence of BS varies from 2.4 to 18% with equal sex distribution.<sup>2-5</sup>

The BS pigment is made up of a black insoluble ferric compound, probably ferric sulfide deposited on the dental surface and formed as a result of the chemical reaction between hydrogen sulfide, produced by chromogenic bacteria (anaerobic bacteria) and

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the iron present in the saliva; the iron, copper, and sulfur complexes are believed to be responsible for the dark color.

Bacteria, such as, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens*, are among chromogenic anaerobes in the oral cavity. Former studies assumed *Prevotella melaninogenica*, *Tannerella forsythia*, and actinomycetes are also found in BS.<sup>3,6</sup>

Factors, such as, eating habits, socioeconomic status, and iron supplementation can contribute to the formation of BS.<sup>4</sup>

As there are no guidelines for their treatment, BS are normally removed through professional oral hygiene session with ultrasound, air-polishing, and abrasive polishing pastes, but in 30-40 days, they tend to recur in 100% of cases since the professional hygiene session acts only on the esthetic condition but not on the cause that determines its formation.<sup>3-6</sup>

Research and progress in the medical and dental fields are constantly evolving.<sup>7-12</sup> Probiotics, i.e., food supplements based on selected bacteria, are a possible therapeutic strategy in the hands of the dentist or dental hygienist to recolonize oral microflora and

replace the bacteria responsible for the pigmentations thus solving the BS blemishes.

The present pilot study aimed to outline a protocol to reduce the recurrence of BS, using in the test group (group I or group A) the administration of a probiotic-based on *Lactobacillus reuteri* and comparing the results with the control group (group II or group B) composed of patients who received a professional oral hygiene session and a motivational upgrade.

The aim of this study was also to verify the effectiveness of *L. reuteri*-based probiotic supply in the selection of new bacterial colonization and to check if, after the elimination of BS through professional oral hygiene maneuvers, it was possible to prevent their recurrence by replacing the chromogenic bacteria present in the oral cavity with new microorganisms, through the introduction of probiotic food supplements.

At the same time, this study aimed to lay the foundations for the continuation of the same project, allowing, in the future, the figure of the dental hygienist to have an additional resource to propose to patients to combat this blemish.

## MATERIALS AND METHODS

All patients were informed about the research and signed informed consent as stated before. The study protocol was approved by the local Ethics Committee and performed according to the declaration of Helsinki.

This pilot study was a randomized controlled trial and was conducted at the Dental Clinic of San Gerardo Hospital in Monza, Department of Medicine and Surgery, during the year 2019.

Twenty patients, 8 males and 12 females, aged between 8 years and 24 years were involved in the study after signing informed consent, consent to the processing of personal data, and a photographic consent and only after having been informed of the treatment to which it would have been exposed. In this study, since most of the study participants examined were people under the age of 18 years, the signatures of the consents were carried out with one or both parents, to whom the study protocol was also explained.

Each study participant had extrinsic pigmentations on one or more teeth, with black lines attributable to chromogenic bacteria in terms of appearance, localization, and anamnestic negativity at all conditions predisposing the formation of other types of pigmentation.

Smokers and people allergic to fine dust were excluded. Similarly, patients allergic or intolerant to glycine or other principles contained in the prophylaxis powder used in the professional oral hygiene session were excluded.

This pilot study took place over a period of 6 months.

To detect the proliferation of BS in teeth, the Lobene modified index was used (Lobene, 1988).<sup>3,13</sup> To use this index, the vestibular surface was divided into two parts: gingival or tooth body (the gingival region is a half-moon-shaped area of about 2 mm wide, adjacent to the margin of the free gingiva that extends to the crest of the interdental papilla of the adjacent tooth).

- The intensity of the pigmentation had a score from 0 to 3:
  - 0: no pigmentation.
  - 1: light pigmentation (from yellow to light brown or gray).
  - 2: moderate pigmentation (intermediate brown).
  - 3: very marked pigmentation (dark brown to black).
- The area of the tooth surface affected by pigmentation had a score from 0 to 3:

- 0: no pigmentation detected.
- 1: pigmentation covers 1/3 of the dental surface.
- 2: pigmentation covers 1/3 to 2/3 of the dental surface.
- 3: the pigmentation covers more than 2/3 of the dental surface.

The data collected through the two indices were then transformed into a percentage.

The pilot study was a randomized controlled trial with the sample divided into two groups randomly: the first (group I, test group) received a professional oral hygiene session and a remark of the oral hygiene education and motivation, followed by a therapy with a probiotic food supply containing *L. reuteri* for a duration of 2 months. The second group (group II, control group) received only a professional oral hygiene session and a remark on oral hygiene education and motivation. The patients were attributed randomly to group I or II. All patients were evaluated by the same operator and 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 (Table 1).

During the first visit (T0), the patient was recruited, the visit was performed to evaluate the possibility of including the patient in the study, photographic documentation was detected, the presence of BS using the Lobene modified index was quantitatively identified, the professional oral hygiene session was performed, the motivation for home hygiene was reinforced, and finally, if assigned to group I, the probiotic was prescribed with an explanation of the methods of administration (dissolving it inside the oral cavity in the evening after home oral hygiene).

The probiotic food supplement used was ProDentis® (BioGaia, Stockholm, Sweden), a food supplement in the form of tablets, containing the association of two strains of lactic ferments: *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289; each probiotic tablet contains at least 200 million live cells of *L. reuteri*.

The patients were also prescribed not to use any other probiotic or antiseptic.

In the second visit (T1, 30 days after T0 or after the end of the first box of probiotics), photographic documentation was carried out, the assessment of the level of oral hygiene with identification and registration of the presence of BS through the Lobene modified index and all patients were again motivated for home hygiene.

**Table 1:** Lobene modified index in group I at T0, T1, and T2 (test group)

Group I Patient no.	Lobene modified index		
	T0 (%)	T1 (%)	T2 (%)
1	35	0	9
2	19	6	1
3	28	6	0
4	8	0	0
5	31	1	10
6	22	5	5
7	48	20	9
8	12	0	6
9	7	1	1
10	36	8	3
Mean	24.6	4.7	4.4

In the third visit (T2), performed after 60 days from the beginning of the therapy, the last check with photographic collection and recording of the Lobene modified index was carried out.

### STATISTICAL ANALYSIS

The data collected during the three visits were then subjected to statistical analysis with Social Science Statistic software.

To analyze the data, the averages of the data collected from the two groups were calculated so that they could be compared.

The attention focused more on the comparison of the Lobene modified index since it was the parameter that has allowed to evaluate the effectiveness of the treatment, as it analyzed the pigmentation qualitatively and quantitatively.

Kolmogorov–Smirnov’s test allowed to determine the absence of a normal distribution of values. The significance of the change in the Lobene modified index between T0 and T2 was performed using the Wilcoxon test for paired data. The difference in T2 between the control group and the test group was determined by the Mann–Whitney *U* test for independent samples.

Differences with  $p < 0.05$  were considered statistically significant.

### RESULTS

In group I, it could be noticed that at T0 the modified Lobene index had values between 7 and 48%, with a mean value of 24.6%; at T1 between 1 and 20%, with a mean value of 4.7% and at T2 between 0 and 9%, with a mean value of 4.4%.

In group II, the average between T0 and T1 remained almost constant, with an increase in T2 compared to T0 (Table 2).

The mean Lobene index was lower in the test group compared to the control group both in T1 and T2. For the statistical analysis between the two groups, the situation was compared to T1 and T2 using the Mann–Whitney test for unpaired data, with the result that the difference in the averages of the Lobene index between the test group and the control group at T1 was statistically significant for  $p < 0.05$  (Table 3). Also in T2, the difference in the averages of the Lobene index between the test group and the control group was statistically significant for  $p < 0.05$ .

By comparing the modified Lobene index in T2 with respect to T0 in group I through the Wilcoxon test for paired data, it emerged that the difference was statistically significant with a  $p$  value  $< 0.05$ .

### DISCUSSION

The term probiotic was defined by the International Scientific Association for Probiotics and Prebiotics as the set of live microorganisms which, if administered in adequate quantities, give a benefit to the health of the guest.<sup>14</sup>

In 2002, probiotics were defined by the joint commission of Food and Agriculture Organization-World Health Organization (FAO-WHO) as “live microorganisms, mainly bacteria, safe for humans, which when administered in adequate quantities confer a beneficial effect on the health of the host”.<sup>15</sup>

There are currently eight probiotics considered safe for human beings:

- *L. reuteri*.
- *Lactobacillus casei*.
- *Lactobacillus acidophilus*.

**Table 2:** Lobene modified index in T0, T1, and T2 (control group)

Group II	Lobene modified index		
	T0 (%)	T1 (%)	T2 (%)
Patient			
11	40	35	45
12	30	27	37
13	28	23	68
14	48	42	47
15	53	46	60
16	32	27	36
17	41	36	56
18	43	39	44
19	29	27	33
20	46	45	67
Mean	39.0	34.7	49.3

**Table 3:** Mean Lobene modified index at T1 and T2 for group I and II

Group	T1	T2
I	4.7	4.4
II	34.7	49.3

- *Bifidobacterium lactis*.
- *Bifidobacterium longum*.
- *Saccharomyces cerevisiae*.
- *Carnobacterium maltaromaticum*.<sup>16</sup>

Probiotics intended for humans can be divided into food and pharmaceutical probiotics. The latter are used to treat pathologies of intestinal origin while the former are integrated through nutrition in four different basic ways: as a concentrated culture inside drinks or food (e.g., fruit juice), in prebiotic fibers, in foods milk-based (e.g., milk, yogurt, cheese), as cell concentrates packaged in dietary supplements.<sup>17,18</sup> *Lactobacillus reuteri* is one of the most common probiotic food supplies used in dentistry, this is why the authors wanted to test it in the prevention or treatment of the patient with BS.

The general mechanism of action of traditional probiotics concerns the normalization of the intestinal bacterial flora, the modulation of the immune response, and has metabolic effects; the mechanism of action of probiotics in the oral cavity is similar. To carry out their action, probiotics must be able to resist oral conditions, resist environmental defense mechanisms, adhere to the saliva-coated surfaces, colonize the mouth, and proliferate to inhibit oral pathogens.<sup>17,19–21</sup>

Only when probiotics are exposed to saliva their survival and resistance to environmental factors in the mouth can be assessed. Salivary proteins, such as, lysozyme, lactoferrin, histatin, salivary peroxidase, and IgA can influence the vitality of probiotics or the morphological characteristics, thus influencing their adhesion to oral surfaces and metabolic activity.<sup>22</sup>

The use of probiotics is a relatively undeveloped strategy for the prevention and treatment of BS, periodontitis, and caries. Their action is carried out by creating conditions that are not favorable to the pathogen to take hold in the intestinal tract, rather than directly inhibiting the growth or vitality of the pathogen. They can alter the ability of the pathogen to adhere or invade the host, they can modify the gene expression program of the pathogens in such a way as to inhibit the expression of the virulent effect and



can create an unfavorable environment for pathogens by altering the pH, the mucus layer, and other local factors.<sup>15</sup>

The action of probiotics is therefore based on the modifications of the bacteria present in the biofilm, interfering with the growth and development of biofilm and replacing microorganisms with beneficial bacteria thus preventing colonization by periodontal pathogens or BS-related bacteria through competition for nutrients or sites of adhesion.<sup>23</sup>

It is thanks to the above-mentioned bacterial competition that in the present study the authors were able to explain the results obtained. Already after 30 days (T1), the difference between the two groups analyzed was evident in terms of pigment reduction, as evidence of the reduction of the black staining microorganisms and their partial replacement with *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 (Fig. 1).

It is interesting to note that during the last visit (T2) the modified Lobene index compared to T0 was lower for 100% of patients in a statistically significant way with  $p < 0.05\%$  (Fig. 2).

The modified Lobene index in group I is reduced statistically significantly compared to the first visit already in T1. However, it can be assumed that despite this reduction, 30 days (T1) is a too short period to suspend the probiotic food supply, since a further

month of administration further reduced the average values of the modified Lobene index (from 4.7 to 4.4).

As can be seen from graphic 3, a microbiological repopulation was carried out through the introduction of a probiotic food supply which led to the elimination and absence of recurrence of the microorganisms causing BS in 60 days (Fig. 3).

It can be seen in the control group how, after an initial decrease in the modified Lobene index 30 days after the oral hygiene session demonstrating the short-term effectiveness of the latter, it increases again at 60 days, confirming the difficulty in preventing the recurrence of BS as also shown by studies in the literature<sup>2-5,14,24</sup> (Fig. 4).

Only two studies could be found in the literature regarding the effectiveness of probiotic food supply in reducing the reformation of BS in children and young adults. Gobbi et al. evidenced *in vitro* an antagonistic ability of *Streptococcus salivarius* M18 and *L. reuteri* to reduce the growth of microorganisms associated with black tooth stains.<sup>24</sup> The effectiveness of an *S. salivarius*-based probiotic in preventing BS formation was also tested *in vivo*, whereas after 6 months BS were detected in 9 out of the 28 children (32.1%) from the test group compared to 14 on 26 (53.8%) from the control group; in this study, however, the presence of BS was recorded only as "present" or "absent".<sup>25</sup>

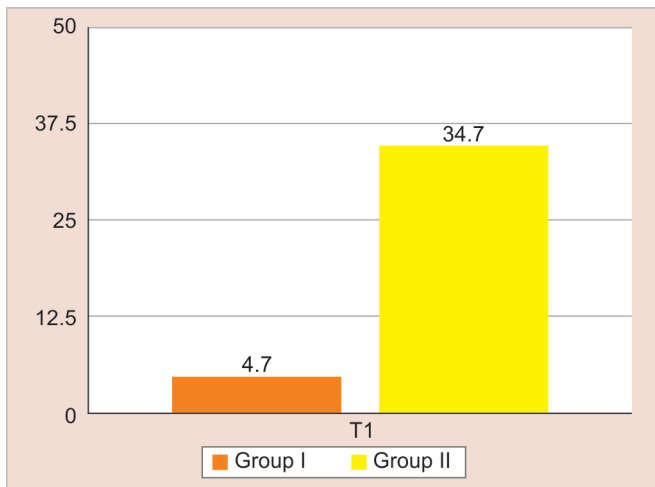


Fig. 1: Lobene index at T1 in group I and II

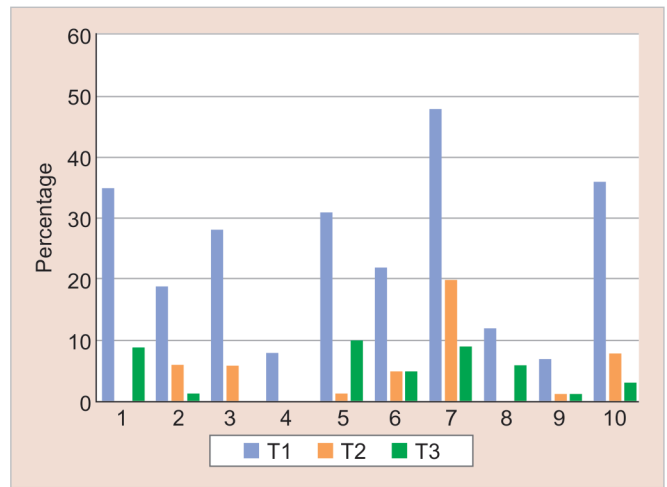


Fig. 2: Test group: changes of Lobene modified index



Fig. 3: Lobene modified index in control and test group from T0 to T2

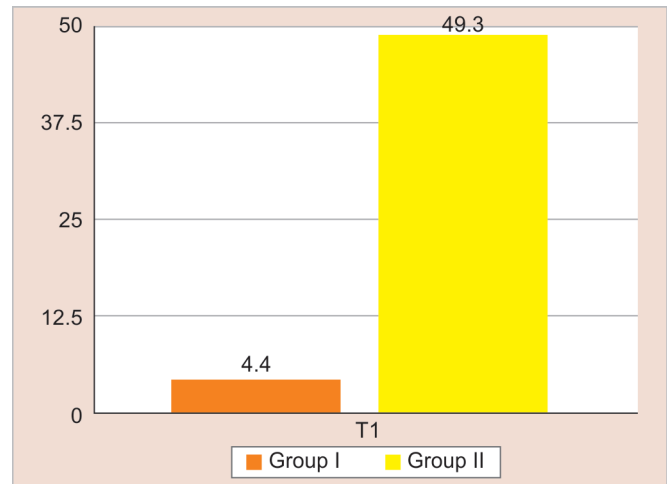


Fig. 4: Lobene index at T2 in group I and II



The use of probiotics is extended to different fields of dentistry. Among them, the improvement of periodontal health, the possible control of caries, halitosis, candidiasis, the prevention of peri-implant disease, and the reduction of enamel demineralization.<sup>11,12,15,16,26-32</sup>

The Center for Disease Control (CDC) supported in 2017 the conclusion that the incidence of *L. reuteri* bacteremia is inconsistent and its oral use is safe.<sup>33,34</sup>

Also from other studies conducted on the research for side effects of *L. reuteri* no adverse effect or contraindication to the intake of probiotics has emerged.<sup>35-38</sup>

Colonization of the oral cavity is selective and specific for each site which is more or less stable over time. However, it must be considered that the introduction of allochthonous bacteria, even in large numbers, is usually followed by their rapid elimination, so the effect of the probiotic may decline over time.<sup>19,39-43</sup>

## LIMITATION OF THE STUDY

A limit of this study was that the control group did not receive a placebo tablet. Although the nature of the antimicrobial activity exerted by the probiotics in this work remains unclear, *L. reuteri* can be considered a good nutraceutical way to reduce BS recurrence. Further studies will also investigate its biochemical mechanism.

## CONCLUSION

In the present study, it was possible to confirm, as found in the literature, that BS pigmentation has a recurrence, variable over time, as found in group II study participants who underwent only professional oral hygiene.

This experimental study shows that the presence of the dark pigmentations assessed using the modified Lobene index after 2 months improves if treated with probiotic, compared to a control group where only professional oral hygiene session was applied. So the professional hygiene session and careful education and motivation, together with a probiotic-based on *L. reuteri* (ProDentis®, BioGaia) to be used once a day for a month, is valid support in the prevention of recurrence from BS.

Furthermore, it is important to highlight that this type of treatment, unfortunately, does not eliminate definitely BS, but still allows us to reduce its extension and intensity, therefore decreasing its recurrence.

More prolonged studies over time are therefore necessary, to assess in detail the presence of any relapses in patients who have taken ProDentis® and to identify different treatment durations that could be more effective in preventing relapses.

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