Effect of the probiotic *Lactobacilli reuteri* (Prodentis) in the management of periodontal disease: a preliminary randomized clinical trial

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Objectives: The aim of this study was to evaluate the effects of *Lactobacilli reuteri* (Prodentis) alone and in combination with scaling and root planing (SRP) in a double blind, randomized, placebo-controlled clinical trial of volunteers with chronic periodontitis.

Methods: Thirty, otherwise systemically healthy, chronic periodontitis patients (19 males and 11 females, aged between 34 and 50 years) were included. The study period was 42 days. ‘Split-mouth’ design was used for the SRP, which was performed on day 0; two quadrants (either right or left) were treated with SRP whereas the remaining two quadrants were left untreated. The participants received a toothbrush, toothpaste, and brushing instructions. *L. reuteri* Prodentis lozenges (1 × 10⁸ CFU DSM17938 + 1 × 10⁸ CFU ATCC PTA 5289) or the corresponding placebo lozenges were taken twice daily from day 21 to day 42. Statistical analysis was done for comparisons of clinical parameters (Plaque Index (PI), Gingival Index (GI), Gingival Bleeding Index (GBI), probing pocket depth (PPD), clinical attachment level (CAL)) and microbiological levels of the pathogens *Aggregibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), and *Prevotella intermedia* (*Pi*). All p-values less than 0.05 were considered significant. Assessments were made on day 0 before SRP treatment, on day 21 before administration of the lozenges, and on day 42.

Results: At day 42, the PI, GI, and GBI were significantly reduced by all treatment modalities. When ranked, the amount of PI, GI and GBI reduction by the different treatments was SRP + Prodentis > Prodentis > SRP + placebo > placebo; all differences were statistically significant. For PPD and CAL, the best result was obtained with the SRP + Prodentis treatment. PPD was reduced from 5.08 ± 0.75 to 3.78 ± 0.61 mm (p < 0.001) and CAL from 3.93 ± 0.93 to 2.85 ± 0.74 mm (p < 0.001). Prodentis, either alone or following SRP, reduced *Aa*, *Pi*, and *Pg* by 1 log₁₀ unit (p < 0.01). The SRP + placebo combination did not significantly affect the levels of the pathogens.

Conclusion: The present randomized controlled trial confirms the plaque inhibition, anti-inflammatory, and antimicrobial effects of *L. reuteri* Prodentis. *L. reuteri* Prodentis probiotic can be recommended during nonsurgical therapy and the maintenance phase of periodontal treatment. Considering the beneficial effects of probiotics, this therapy could serve as a useful adjunct or alternative to periodontal treatment when SRP might be contraindicated. Further studies are required in this direction.

Keywords: scaling and root planning; DSM 17938; ATCC PTA 5289; chronic generalized periodontitis; anti-inflammatory; antimicrobial; plaque; gingivitis

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There is a long tradition, particularly in parts of Europe and Asia, of ingesting microbes or food products that affect the intestinal microbiota in ways that are believed to provide beneficial health effects, i.e. intake of probiotics and prebiotics. According to the World Health Organization, probiotics are defined as viable microorganisms that confer a health benefit when administered in sufficient doses.

A few studies have revealed that probiotic *Lactobacillus* strains were useful in reducing gingival inflammation and
Written informed consent was obtained from all patients. Patients of both sexes within the age limit of 35–50 years were eligible for inclusion. Patients diagnosed as suffering from chronic periodontitis as clinically evidenced by gingivitis together with mild to moderate periodontal pockets (5–7 mm) clinically and radiographic evidence of bone loss were included (10). Patients who were pregnant, lactating, smokers, alcoholic, or who had undergone any surgical or non-surgical therapy within 6 months prior to the start of the study were not included. Written informed consent was obtained from all patients.

All subjects were assigned to one of the two groups (Prodentis group or placebo group) by using a randomization table. To maintain full blinding of the results, the randomization code was held by one of the authors remotely from all assessments and was not broken until all data had been collected and all analyses had been performed. The randomization was concealed by using sequentially numbered, identical-appearing containers of probiotic or placebo tablets.

Clinical and microbiological parameters were recorded at baseline (day 0), on day 21, and day 42. The clinical parameters recorded were PI (11), GI (12), GBI (13), probing pocket depth (PPD), and clinical attachment level (CAL) (14). PPD and CAL were measured for the indexed teeth of the Community Periodontal Index using the WHO probe (14). All parameters were assessed by a single clinical investigator experienced with the index systems.

Material and methods
This was a randomized, placebo-controlled, double blind, split-mouth designed clinical study to evaluate the effect of probiotic lozenges with and without SRP on the clinical parameters and microbiological profile in subgingival plaque samples of chronic periodontitis patients.

To calculate the proper sample size, the change in probing depth at the end of 42 days was estimated to be 0.1 mm in the test group. The sample size was calculated to be approximately 15 subjects each in the test and placebo groups with a power of 0.80 and α at 0.05.

Patients of both sexes within the age limit of 35–50 years were eligible for inclusion. Patients diagnosed as suffering from chronic periodontitis as clinically evidenced by gingivitis together with mild to moderate periodontal pockets (5–7 mm) clinically and radiographic evidence of bone loss were included (10). Apart from having chronic periodontitis, the patients were in general good health and had not participated in any clinical trial during the previous 4 weeks. No patient had ongoing antibiotic treatment or any systemic disease. Patients who were pregnant, lactating, smokers, alcoholic, or who had undergone any surgical or non-surgical therapy within 6 months prior to the start of the study were not included. Written informed consent was obtained from all patients.

Patients who were undergoing any surgical or non-surgical therapy within 4 weeks. No patient had ongoing antibiotic therapy (10). Apart from having chronic periodontitis, the patients were in general good health and had not participated in any clinical trial during the previous 4 weeks. No patient had ongoing antibiotic treatment or any systemic disease. Patients who were pregnant, lactating, smokers, alcoholic, or who had undergone any surgical or non-surgical therapy within 6 months prior to the start of the study were not included. Written informed consent was obtained from all patients.
mediums: enriched trypticase soy agar for Aggregibacter actinomycetemcomitans (Aa) cultivation (15); kanamycin blood agar for Pg (16); and kanamycin and vancomycin blood agar for Prevotella intermedia (Pi) (16). The samples were then vortexed at 3000 x g for 1 min to break the plaque and to obtain uniform dispersal of organisms. The plates were kept under anaerobic conditions. The medium for Aa was incubated in a desiccator with 5% carbon dioxide at 37°C for 5 days. The plates were incubated for a minimum of 72 h. After the specified period, the colonies were characterized according to size, shape, hemolysis, and pigmentation and the number of each colony type was counted.

Aa was identified by its characteristic star-shaped colonies on the agar plate, Gram negative coccal bacillary morphology, negative indole test, nitrate reduction, and glucose fermentation. Porphyromonas and Prevotella species were provisionally identified by pigment production, colony characters, cell morphology, and the variation in their susceptibility to kanamycin and vancomycin. The identity of these organisms was further confirmed by subjecting them to a series of biochemical reactions, which included indole production, nitrate reduction, and sugar fermentation tests. The sugars tested were dextrose, mannitol, lactose, sucrose, maltose, salicin, glycerol, xylose, arabinose, trehalose, rhamnose, and xylan. A uniform standardized suspension of the organisms was prepared in thioglycollate broth and inoculated into peptone yeast extract broth containing 1% of the appropriate sugar and bromocresole purple as the indicator. The tubes were incubated for 48 h in a modified gas-pak anaerobic jar and the fermentation reactions were noted by a change in the color of the indicator to yellow. Indole and nitrate reduction tests were performed by spot disc method using commercially available reagents (Hi-Media).

All clinical and microbiologic data collected were subjected to statistical analysis. Results are expressed as mean ± SD and proportions as percentages. For clinical parameters, intra-group comparisons were made by paired t-test and inter-group comparison by unpaired t-test. For microbiological parameters, non-parametric methods were used for analysis since microbes were non-normally distributed; the Wilcoxon’s signed rank test was used for intra-group comparison and the Mann–Whitney test for inter-group comparison. For all tests a p-value of less than 0.05 was considered statistically significant.

The study protocol was in accordance with the local ethical guidelines and in accordance with the Helsinki Declaration of Human Rights and approved by the local ethics committee.

Results
Thirty, systemically healthy, chronic periodontitis patients (19 males and 11 females, aged between 34 and 50 years) were included. Age, gender distribution, clinical and microbiological parameters were similar in both groups at day 0 (baseline) (Table 1). All subjects completed the 42-day study period. There were no significant differences in the clinical and microbiological parameters between the Prodentis and placebo groups at day 0.

The active study product of Prodentis lozenges was re-analyzed by the producer (BioGaia AB, Sweden) at the end of the study and the CFU count of both strains was above the stipulated shelf-life limit of 1 x 10^8 CFU. The presence of lactobacilli in the saliva, plaque, or gingival pockets was not assessed in this study.

Within both the Prodentis and the placebo groups, with or without SRP, PI, GI, or GBI, the reduction was highly significant (p<0.001) over the treatment periods (Fig. 1). The maximum reduction of PI (0.76 ± 0.29) was obtained for the combination of SRP and Prodentis, which was statistically significant when compared to all other treatment modalities (p<0.01) (Table 2). In the untreated quadrants, Prodentis was significantly better than the placebo with a reduction of 0.41 ± 0.16 and 0.17 ± 0.14, respectively (p<0.001). Also for the GI and GBI, the best reduction was obtained with the combination of SRP and Prodentis as compared with the other treatment modalities (p<0.001), the GI fell from 1.85 ± 0.22 to 1.01 ± 0.10 and the GBI from 81.6 ± 18.4 to 11.1 ± 4.6% over the full period. In the untreated quadrants, Prodentis was better than the placebo on both accounts, the GI was reduced by 0.53 ± 0.12 and 0.14 ± 0.14 units (p<0.001) and the GBI by 48.3 ± 14.4 and 12.0 ± 8.7 percentage units (p<0.001), respectively. An interesting observation was that Prodentis alone demonstrated a highly significant (p<0.001) GBI reduction as compared to SRP alone.

In the untreated quadrants, the PPD was only slightly changed after the placebo or Prodentis treatment, however these changes were not significant either over time or between groups. Over the 42 days, SRP alone

Table 1. Baseline characteristics of study participants (mean (SD))

|                     | Prodentis group | Placebo group | Significance |
|---------------------|-----------------|---------------|--------------|
| Men/women           | 9/6             | 10/5          | ns           |
| Age (years)         | 41.4 (5.3)      | 41.5 (4.9)    | ns           |
| PI (score)          | 1.79 (0.36)     | 1.77 (0.20)   | ns           |
| GI (score)          | 1.85 (0.22)     | 1.88 (0.12)   | ns           |
| GBI (%)             | 81.6 (18.4)     | 87.9 (13.5)   | ns           |
| PPD (mm)            | 5.08 (0.75)     | 5.26 (0.53)   | ns           |
| CAL (mm)            | 3.93 (0.93)     | 4.46 (1.94)   | ns           |
| Aa (CFU/ml x 10^6) | 105.3 (66.8)    | 103.0 (66.4)  | ns           |
| Pg (CFU/ml x 10^6)  | 89.7 (70.4)     | 98.7 (60.4)   | ns           |
| Pi (CFU/ml x 10^6)  | 81.0 (67.0)     | 80.3 (73.1)   | ns           |
Fig. 1. Results of the clinical assessments during the study period. Error bars indicate SD. ■=Prodentis group; □=placebo group.
significantly reduced PPD from 5.26 ± 0.53 to 4.77 ± 0.60 mm (p < 0.001). The maximum PPD reduction was observed in SRP+Prodentis treatment from 5.08 ± 0.75 to 3.78 ± 0.61 mm, which was significantly better than SRP alone (p < 0.001).

The CAL did not change significantly over time in the untreated quadrants group receiving placebo whereas there was a small decrease in the corresponding Prodentis group from 3.85 ± 1.44 to 3.67 ± 1.31 mm (p < 0.05), which was also better than for the placebo group (p < 0.05). The SRP+placebo treatment yielded a decrease from 4.46 ± 1.94 to 4.17 ± 1.82 mm (p < 0.05). Again, the best result was obtained for the combination of SRP+Prodentis where the CAL dropped from 3.93 ± 0.93 to 2.85 ± 0.74 mm (p < 0.001). This was also better than any other treatment modality (p < 0.001).

The only treatment modalities that were able to significantly reduce the CFU counts of the pathogens Aa, Pg, and Pi were those including Prodentis, either alone or in combination with SRP (Table 3 and Fig. 2). All three pathogens were equally sensitive toward Prodentis treatment and bacterial counts were reduced by approximately 1 log10 unit from 106 CFU/ml to less than 105 CFU/ml.

Compliance to the study treatment was appreciable and no subject rejected using the lozenges. Hypersensitivity as a symptom of root caries was not reported by any of the study subjects and no other adverse reactions were reported by the subjects or observed by the clinician during the study period.

Discussion

The effect of SRP as a non-surgical mode of periodontal therapy is well discussed in the literature in terms of a reduction of clinical and microbial parameters. According to a review in 2009 (17), the basic/initial treatment for periodontal patients in terms of SRP has not been compared with the probiotic effect in the reduction of clinical and microbiological parameters. Hence, an initial attempt has been made in this randomized, double blind clinical trial to evaluate and compare the benefits of the
Pi Krasse et al. (2) conducted a study to assess if the no difference in mean plaque reduction was observed. Enhanced by the use of probiotics. Probiotics and SRP thus the plaque reduction brought about by SRP was when compared to SRP and probiotic effects individually, Prodentis demonstrated a significant reduction of PI reported by Mousque’s et al. (9) and Proye et al. (20). The effect of SRP on plaque reduction is similar to that reported by Mousques et al. (9) and Proye et al. (20). Mousques reported the reduction of PI and GI during the first 14 days after a single mouth SRP and Proye et al. reported the response of 128 periodontal pockets (3-7 mm) in 10 subjects after a single episode of SRP to give a significant reduction in PI and gingival findings after 1 week.

In the Prodentis group, both the GI score and GBI percentage reduction were significant both with Prodentis alone and in combination with SRP. An interesting observation was that the Prodentis alone group demonstrated a highly significant gingival bleeding reduction as compared to SRP alone. Interestingly, the gingival bleeding reduction by SRP was enhanced by the probiotic. Ma et al. (21) studied the in-vitro effects of live L. reuteri on human epithelial cells and found that L. reuteri is able to block the TNFα-induced secretion of the pro-inflammatory IL-8, upregulate NGF, and inhibit NF-kB translocation to the nucleus. Twetman et al. (6) investigated the clinical effect of a chewing gum containing probiotic bacteria on gingival inflammation and the levels of selected inflammatory mediators in gingival crevicular fluid (GCF) in patients with gingivitis. The chewing gums contained two strains of L. reuteri: ATCC 55730 and ATCC PTA 5289 (1 x 10^8 CFU/gum, respectively). They found that the pro-inflammatory cytokines IL-1β, TNFα, and IL-8 in GCF were reduced by active probiotic treatment, which gives clinical support to the findings by Ma et al. (21). This may be the proof of a principle for a probiotic approach to combating inflammation in the oral cavity and the findings by Twetman et al. (6) are confirmed in the present study as evidenced by the reduced gingivitis parameters (GI and GBI).

There was a significant improvement of PPD and CAL in the Prodentis group, which could possibly be due to the significant reduction in the GI and PI. The maximum PPD reduction was observed in those receiving SRP + Prodentis treatment and this reduction (1.31 mm) was more than twice the sum of the SRP alone reduction added to the Prodentis alone reduction (0.49 + 0.10 mm),

| Group         | Reduction | Group         | Reduction | Significance |
|---------------|-----------|---------------|-----------|--------------|
| Aa SRP + Prodentis | 94.0 (62.8) | SRP + placebo | 6.4 (75.7) | p<0.005      |
| SRP + Prodentis  | 94.0 (62.8) | Prodentis     | 74.7 (67.9) | ns           |
| SRP + placebo   | 6.4 (75.7)  | Placebo      | 10.0 (21.0) | ns           |
| Prodentis       | 74.7 (67.9) | Placebo      | 10.0 (21.0) | p<0.01       |
| Pg SRP + Prodentis | 85.7 (73.5) | SRP + placebo | 0.4 (46.4) | p<0.005      |
| SRP + Prodentis  | 85.7 (73.5) | Prodentis     | 91.3 (51.7) | ns           |
| SRP + placebo   | 0.4 (46.4)  | Placebo      | -6.8 (32.3) | ns           |
| Prodentis       | 91.3 (51.7) | Placebo      | -6.8 (32.3) | p<0.001      |
| PI SRP + Prodentis | 77.0 (65.1) | SRP + placebo | 6.4 (67.9) | p<0.05       |
| SRP + Prodentis  | 77.0 (65.1) | Prodentis     | 71.0 (63.6) | ns           |
| SRP + placebo   | 6.4 (67.9)  | Placebo      | -16.4 (69.2) | ns           |
| Prodentis       | 71.0 (63.6) | Placebo      | -16.4 (69.2) | p<0.001      |

Table 3. Statistical comparison (Wilcoxon’s signed rank test) of the microbiological effects between the treatment modalities after 42 days (values are given as mean (SD) CFU/ml x 10^6).
suggesting a synergistic effect. An improvement was observed for CAL; SRP alone yielded 0.29 mm, Prodentis yielded 0.17 mm and the combination resulted in a CAL improvement of 1.09 mm. It should be noted that to our knowledge this is the first time that Prodentis or any other probiotic treatment has been shown to improve CAL. In a randomized, double-blinded, placebo-controlled study, Shimauchi et al. (5) reported a significant improvement of the PI and PPD in the probiotic group (*L. salivarius* WB21), but CAL was not assessed in their study. Moreover, in their clinical setting there was no significant difference between the probiotic and placebo group in the complete set of patients.

It has been observed that there is a substantial *in vitro* variation in the antimicrobial activity of *Lactobacillus* spp. when examined together with several oral microorganisms, including the periodontal pathogens *Aa* and *Pg* (9). *Aa* strains were the most susceptible species to lactobacilli under the conditions of this experiment. The *in-vitro* effect of probiotics on *Aa*, *Pg*, and *Pi* has been studied (22), and in the present study the antimicrobial effect of *L. reuteri* Prodentis has been tried for the first time in clinical periodontitis cases. The effect of *L. reuteri* Prodentis on salivary mutans streptococci was not addressed in the present study, but as reported by Caglar et al. (31), *L. reuteri* significantly reduces mutans streptococci when administered over a 10-day period.

In the present study, the mean CFU reduction of the three assessed pathogens was highest for the combination of SRP+Prodentis followed by Prodentis alone and

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**Fig. 2.** Results of the microbiological assessments during the study period. Error bars indicate SD. ■ = Prodentis group; □ = placebo group.
the size of the reduction was approximately one log unit. The mechanisms of inhibition of periodontal pathogens have not been fully clarified. The inhibitory activity displayed by homofermentative lactobacilli against periodontal pathogens was principally related to their production of acid, not to \( \text{H}_2\text{O}_2 \) or bacteriocin production (23). In the present study, at baseline the 30 chronic generalized periodontitis patients demonstrated the presence of \( \text{Aa} \), \( \text{Pg} \), and \( \text{Pi} \) in accordance with the findings of Socransky et al. (23). The results obtained by Mousque`s et al. (9) suggest that a single session of SRP is capable of disturbing the proportions of certain bacterial forms in the subgingival periodontal flora, and that it may require approximately 42 days for the proportions to return to baseline levels. Studies designed to determine the effect of SRP on the subgingival microbial flora have consistently reported significant reductions in the percentage of motile microbes and spirochetes (9, 22, 23), \( \text{Pg} \) and other Gram negative anaerobic bacteria and a concomitant increase in the percentage of cocci and non-motile bacteria (9, 24, 25).

In contrast to the obtained effects of SRP alone on the clinical parameters, we found no significant effects of SRP alone on the microbiological parameters. However, this phenomenon has also been reported by others. Sbordone et al. (26) evaluated the recolonization patterns of the subgingival microflora of eight adult periodontitis patients after a single session of SRP. Their results indicate that a single session of SRP is clearly insufficient to maintain a healthy subgingival microflora. Mombelli et al. (27) conducted a study to determine the topographic distribution of \( \text{Aa} \) in patients with adult periodontitis before and after mechanical periodontal treatment (repeated oral hygiene instructions and systematic deep SRP) and found that \( \text{Aa} \) was present in 40% of the samples taken before and in 23% of the samples taken after treatment. Persistence of the organism after thorough debridement of root surfaces has also been reported (27).

Both the \( \text{L. reuteri} \) strains used in the present study produce the antimicrobial substance reuterin, 3-hydroxypropionaldehyde, within biofilms (28, 29) and it was recently demonstrated that reuterin induces oxidative stress in pathogenic organisms (29), which accounts for its anti-pathogenic effect. Reuterin is effective against a vast array of intestinal pathogens (30, 31) and the present study suggests a similar effect against selected periodontal pathogens. To our knowledge, the present study is the first of its kind to demonstrate the \textit{in vivo} antimicrobial effect of \( \text{L. reuteri} \) Prodentis in a clinical setting.

The present randomized controlled trial confirms the plaque inhibition, anti-inflammatory, and antimicrobial effects of \( \text{L. reuteri} \) Prodentis. Hence, the \( \text{L. reuteri} \) Prodentis probiotic is suggested as an addition to mechanical debridement and during the maintenance phase of periodontal treatment. Further randomized controlled clinical trials over longer periods are required to build up stronger evidence for probiotic application of \( \text{L. reuteri} \) Prodentis supporting a periodontal treatment protocol.

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Conflict of interest and funding

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