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The effect of a streptococci containing probiotic in periodontal therapy: a randomized controlled trial

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Abstract

Aim: To evaluate the adjunctive effects of a *Streptococcus oralis* KJ3, *Streptococcus uberis* KJ2 and *Streptococcus rattus* JH145 containing probiotic tablet after scaling and root *planing* (SRP).

Materials and Methods: Forty-eight periodontitis patients were included in this double-blind, placebo-controlled clinical trial. After root planing, patients used either a placebo or a probiotic tablet twice a day for 12 weeks. The pocket probing depth (primary outcome measure), bleeding on probing and relative attachment levels were measured at baseline, 12 and 24 weeks. At baseline, 4, 8, 12 and 24 weeks, microbiological sampling was performed and plaque and gingival indices were recorded.

Results: The primary and secondary outcome measures were significantly ($p < 0.05$) improved at the 12- and the 24-week evaluation in both groups. However, no significant inter-group differences could be detected at any time point, except from the % of sites with plaque that were significantly lower in the probiotic group than in the control group at the 24-week evaluation. In addition, at the 12-week time point, the salivary *Prevotella intermedia* counts were significantly lower in the probiotic group.

Conclusions: No differences were detected when comparing the adjunctive use of a placebo or the investigated streptococci containing probiotic tablet after SRP. ClinicalTrials.gov Identifier: NCT02403960.

In a susceptible host, the presence of periodontopathogens and the absence of beneficial bacteria are key factors associated with the development of periodontal diseases (Slots & Rams 1991, Socransky & Haffajee 1992, Wolff et al. 1994). Interfering with host susceptibility is difficult, therefore today's used treatment strategies for combating periodontal diseases focus on reducing the pathogenic bacteria (Salvi & Lang 2005) by improvement of the patients' oral hygiene and mechanical subgingival debridement (sometimes supplemented with antimicrobial aids) (Haffajee et al. 2003). Recently, there is an increased interest in restoring the reduced number of beneficial bacteria by the use of probiotics. Probiotics are defined as "living microorganisms which, when administered in adequate amounts, confer a health

benefit for the host" (http://who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf).

At this moment, clinical trials in the periodontal field describe mainly the use of lactobacilli containing probiotics. The positive effects of these have been repeatedly shown (Krasse et al. 2006, Riccia et al. 2007, Shimauchi et al. 2008, Staab et al. 2009, Twetman et al. 2009, Vivekananda et al. 2010, Szkaradkiewicz et al. 2011, Shah et al. 2013, Teughels et al. 2013, Vicario et al. 2013, İnce et al. 2015, Tekçe et al. 2015). However, since streptococci are much more abundant in the oral cavity and are shown to re-colonize the periodontal pockets soon after SRP (Jünemann et al. 2012), probiotic products containing streptococci might be a more valid treatment option. Surprisingly, studies describing the use of streptococci as probiotics for oral health are scarcer. There are some animal studies and few in vivo studies (Burton et al. 2005, 2006a,b, 2011, 2013, Teughels et al. 2007, Hillman et al. 2009, Zahradnik et al. 2009).

Though, at present, there is no randomized controlled clinical trial available that investigated the influence of *Streptococcus* containing probiotics as an adjunct to non-surgical periodontal therapy [scaling and root planing (SRP)].

Therefore, the aim of this study was to examine the additional effects in adult periodontitis patients of the use of a probiotic tablet for 12 weeks containing *Streptococcus oralis* KJ3, *Streptococcus uberis* KJ2 and *Streptococcus rattus* JH145 after SRP compared to SRP and a placebo tablet.

Material and Methods

This double-blind, placebo-controlled, randomized (1:1 ratio) clinical trial with two parallel arms involved 48 patients with advanced adult periodontitis. They were recruited at the Periodontology Department of the Cukurova University, Turkey. All patients referred for periodontal treatment were screened for eligibility. Inclusion criteria were as follows: (i) systemically healthy, (ii) at least 36 years of age, (iii) a minimum of three natural teeth in every quadrant

and (iv) untreated moderate to severe adult periodontitis (Van der Velden 2005). Exclusion criteria were as follows: (i) received antibiotics for any purpose within 6 months prior to entering the study or suffering from a disease condition that would typically require antibiotic prophylaxis before dental treatment, (ii) a history of diabetes, rheumatic fever, liver or kidney disease, neurological deficiencies, or use of medication which may affect periodontal tissue (for example: phenytoin, cyclosporin, nifidepine, chronic use of non-steroidal anti-inflammatory drugs), (iii) pregnancy, (iv) acute oral lesions or necrotizing ulcerative periodontitis and (v) dental personnel. The project was approved by the ethical committee for clinical trials of the Cukurova University in Turkey with number CUDHF-EK-2009-7. No changes in the trial design were made after approval by the Ethical Committee. The trial was registered at ClinicalTrials.gov with the number NCT02403960.

Sample size calculation and randomization

A power analysis prior to the start of the study was difficult since no previous randomized controlled trials on this study product were available. The sample size was at that time determined based on the study of Vivekananda et al. (2010). When probing pocket depth (PPD) was taken into account as primary outcome measure, with an expected difference of 0.82 mm and a standard deviation of 0.61 mm, it was calculated that 10 patients were needed in each group to provide 80% power with an alpha of 0.05 (version 2.7.3; StatsDirect, Cheshire, UK). Despite this low number and taking into consideration studies comparing the adjunctive effect of antibiotics to SRP, it was decided to include 24 patients in each group.

Randomization of the patients was done by block randomization (version 2.7.3; StatsDirect). The study coordinator (MCH) distributed the coded bottles to the examiner (OO) at baseline, 4 and 8 weeks visit. Except for the study coordinator, all patients and study personnel were blinded to the study group allocation. Before send-

ing the data to the biostatistician, the code was broken to group the patients to the proper groups.

Treatment protocol

Patients fulfilling the eligibility criteria were asked to participate in the study and, after approval, to sign an informed consent. Baseline examination included full-mouth PPD, gingival recession (REC) and bleeding on probing (BOP) measured at six sites per tooth. In addition, the plaque (Silness & Loe 1964) and gingival indexes (Loe & Silness 1963) (PI and GI) were recorded. After baseline periodontal examination and microbial analysis, an oral hygiene instruction was given (toothbrush, inter-dental brush). Initial periodontal therapy consisted of a full-mouth one-stage disinfection approach (Quirynen et al. 2006). All clinical procedures were performed by the same periodontist (EY) who was not informed about the group allocation. The patients were asked to rinse for 2 min. with a 0.1% chlorhexidine (CHX) solution (Eludril®; Fabre Medicament, Castres, France). SRP was performed on two consecutive days using an ultrasonic scaler (EMS, Nyon, Switzerland) under 0.12% CHX irrigation (Oroheks; TriPharma, Istanbul, Turkey) and with hand instruments. Local anaesthesia was applied for the comfort of the patients. All mucosal surfaces were afterwards disinfected with CHX on a swab and the tongue was brushed with a CHX gel for 1 min. Afterwards, the participants were randomized over the two treatment groups: control (SRP) or probiotic group (SRP + P). The participants of the probiotic group were asked to let a probiotic tablet dissolve on their tongue twice a day for 3 months. The participants in the control group were asked to do the same with a placebo tablet. All patients were instructed to use the tablets after brushing their teeth in the morning and in the evening. The probiotic and placebo tablets were identical in shape, texture, taste and composition. In addition for the probiotic tablet *S. oralis* KJ3, *S. uberis* KJ2 and *S. rattus* JH145 (Probiora3,;Oragenics, Alachua, FL, USA) were added (at least 10^8 CFU of each strain/tablet). All patients were

supplied with the same toothpaste (Colgate Total®; Colgate-Palmolive, Istanbul, Turkey). They were asked not to use any probiotic containing products during the course of the study. Neither was it allowed to use drugs with anti-inflammatory properties, CHX or other mouth rinses during the study.

At designated time points, follow-up visits were planned. Four and 8 weeks after initiation of the therapy, clinical evaluation (PI and GI) and microbial sampling were performed. Twelve and 24 weeks after the initial treatment all baseline parameters were recorded (PPD, REC, BOP, GI, PI, microbial parameters). All data were recorded by the examiner (OO) who was not informed about the group allocation.

Outcomes variables

Primary outcome measures

The primary outcome measure was PPD. All examinations were performed with a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). A sub-analysis was performed taking into account the initial PPD. A pocket was considered moderate if its initial PPD was between 4 and 6 mm and deep if the initial PPD was ≥7 mm.

Secondary outcome measures

The secondary outcome measures were REC, clinical attachment level (CAL), BOP, GI, PI and microbial parameters. CAL was calculated as the sum of the PPD and REC. The plaque index was noted according to Silness & Loe (1964) and the gingival index according to Loe & Silness (1963) at six sites per tooth. These data were processed dichotomous: when the gingivitis index was not zero, this was called gingivitis, when the plaque index was different from zero it was supposed that plaque was present. "Risk for disease progression" was defined at patient level according to Lang & Tonetti (2003) as low (≤4 sites with PPD ≥ 5 mm), moderate (5–8 sites with PPD ≥ 5 mm) or high (≥9 sites with PPD ≥ 5 mm). The "need for surgery" outcome measure was calculated according to Cionca et al. (2009). A site was considered as "in need for surgery" if the PPD was ≥6 or 5 mm and BOP positive. A tooth was considered in need

for surgery if it had at least one site in need for surgery. A patient was considered in need or surgery if at least one tooth was in need for surgery.

Sub-analyses were performed for CAL and "need for surgery" data taking into account the initial PPD at the same way as described for PPD.

Microbiological samples were collected from supragingival and subgingival plaque, saliva and the tongue. Pooled supragingival plaque samples were taken with Gracey curettes at the four single-rooted teeth with the deepest initial pocket in each quadrant. Before sampling, the sites were isolated from saliva with cotton rolls and then dried with compressed air. All supragingival plaque from these sites was dispersed in 0.75 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). An equal amount of 0.5 M NaOH was added to each Eppendorf tube. Samples were dispersed using a vortex mixer and immediately frozen at −20°C until analysis. On the same teeth, pooled subgingival plaque samples were collected with two paperpoints (#35; Dentsply Maillefer, Ballaigues, Switzerland) per site. These were inserted (one mesial, one distal) until resistance was felt. After 10 s, the paperpoints were transferred to a sterile Eppendorf tube and processed as described above. Saliva samples were obtained by collecting 1 ml unstimulated saliva in a sterile cup. Finally the biofilm of the tongue was collected with a cotton swab. These were wiped 10 times over the tongue starting from the tongue dorsum.

Examiner calibration

The calibration of the examiner (OO) was done on 10 periodontitis patients which were not included in the study, by measuring one quadrant with at least six teeth. PPD and CAL were measured in the given quadrant, after 1 h the same quadrant was measured again. The intra-examiner was accepted if measurements were similar to the millimetre at the >90% level.

Compliance and adverse effects

The patients returned the bottles containing the tablets at the 4-, 8-

and 12-week visit to check for compliance. At each control visit the examiner (OO) questioned the patient in relation to general health changes, use of anti-inflammatory drugs, use of mouth rinses, compliance of the use of probiotic products and any adverse events that the patients might have noticed.

Statistical analysis

Summary statistics that were calculated for continuous variables included number of data, average, median, standard deviation, minimum and maximum. For binary variables the number and frequency of positives was recorded.

Confirmatory analyses were performed on site, tooth and patient level. For analysis on tooth and patient level, averages were calculated per tooth and patient respectively.

Continuous variables were fit with a linear mixed model with treatment as a fixed factor. For analysis on site level, tooth and patient were modelled as random factors. For analysis on tooth level, patient was modelled as random factor. Analyses on patient level were performed with an analysis of variance (ANOVA) with place, treatment and time as random factors. Binary variables where fit with a generalized linear mixed model using the probit function as link function, choice of fixed and random factors was similar to the choice for the continuous variables.

For binary variables, an extra analysis was fit on tooth and patient level where the outcome was the maximum value recorded for the sites for the corresponding tooth or patient. Choice of random factors was performed as above. Comparisons between treatments were performed for each place and time separately. For each time, difference between time points and place, a separate analysis was performed.

For all measurements statistical significance was set as $p \leq 0.05$, when $p \leq 0.1$ to >0.05 this was described as "tendency".

p-Values were corrected for simultaneous hypothesis testing according to Sidak, such that the global significance level for all comparisons for a certain outcome parameter or bacteria was set at 0.05.

Results

The flow chart of the people screened and included in the study is shown in Figure S1. The subjects were recruited from June 2010 up to November 2010. All included participants completed the prescribed treatment. There were 26 males and 22 females included with an age of 37–58 years (Table 1). Based on the returned bottles, the compliance of the participants to the treatment protocol was good (not more than a 5% difference between the expected and the returned number of tablets). No adverse events were reported.

Primary outcome measure: PPD

The primary outcome measure, PPD, is shown in Table 2 and expressed as mean and standard deviation. Looking at the overall PPD a significant decrease in PPD is detected in both the probiotic and the control group, when comparing both the 12- and 24-week measurements with the baseline data. When comparing the 24-week evaluation with the 12-week results, there is an additional, but not significant, decrease in pocket depth. Looking at the inter-group differences, no significant differences between both groups were found at baseline, at 12 and at 24 weeks.

A sub-analysis for moderate (initial PPD between 4 and 6 mm) and deep (initial PPD ≥ 7 mm) pockets was performed to take a closer look at these variables. This sub-analysis showed similar results as the analysis for the overall PPD. In both groups, the pockets were significantly reduced at 12 and 24 weeks when compared to baseline. When the 24-week results were compared to the 12-week results, no significant differences were found for the moderate

pockets. However, for deep pockets there was a statistically significant additional decrease in probing pocket. There were no significant inter-group differences found at any of the evaluation moments.

Based on the PPD data, the "risk for disease progression" according to Lang & Tonetti (2003) was calculated (Table S1). No significant differences between both groups could be detected, neither at the 12-week, nor at the 24-week evaluation.

Secondary outcome measures

CAL, REC, BOP

Clinical attachment level, REC and BOP data are shown in Table 2. Significant intra-group differences can be noted for the control and the probiotic group when CAL measurements at 12- and 24-weeks are compared to baseline measurements. However, no inter-group differences could be found at any of the time points. Similar observations were made for REC and BOP measurements.

When the "need for surgery" is calculated based on PPD and BOP (Table S2), the "need for surgery" at site, tooth and patient level is significantly decreased at 12 and 24 weeks when compared to baseline. However, no significant differences between the control and probiotic group can be noticed after treatment.

PI and GI

The percentage of plaque and gingivitis is presented in Table 3. Concerning the percentage of plaque, all measurements for the probiotic group are significant lower compared with the baseline measurements. This is not the case for the measurements in the control group. Concerning the inter-group differences, the percent-

age of sites with plaque detected was significantly lower in the probiotic group than in de control group at the 24-week evaluation. Concerning gingivitis, the measurements at week 4, 8, 12 and 24 were significantly lower than those at baseline both in the control and the probiotic group. Conversely no significant intra-group differences could be found when examining the percentage of sites with gingivitis, nor at baseline, nor at any follow-up visit. Only at the 24-week evaluation there was a trend towards less sites with gingivitis in the probiotic group compared with the control group.

Microbiological data

The microbiological data for the sub- and supragingival, tongue and saliva samples are presented in Tables 4–7. No data are shown for *Aggregatibacter actinomycetemcomitans* since the vast majority of the obtained data were below the detection limit, which made statistical analysis impossible.

Significant intra-group differences are noticed for *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Tannerella forsythia* when comparing the 4-, 8-, 12- and 24-week data with baseline data. However, no significant inter-group differences could be detected. Except for *P. intermedia* in saliva, for which a significant inter-group difference was found comparing the differences between baseline and the 12-week data ($p = 0.02$). The only trend that could be noted, was the trend that *F. nucleatum* in supragingival plaque decreased more in the probiotic group than in the control group between baseline and the 4-month evaluation ($p = 0.06$).

Discussion

This RCT examined the additional effect of a 12-week use of a probiotic tablet containing *S. oralis* KJ3, *S. uberis* KJ2 and *S. rattus* JH145 compared to a placebo tablet after SRP. For both groups, the PPD was significantly lower at the 12- and the 24-week evaluation when compared to baseline. However, at none of the evaluation moments a significant difference could be detected between the groups. Similar observations where made when analysing this

Table 1. Demographic characteristics

Variable	Treatment group	
	SRP	SRP + P
Number of patients	24	24
Number of males	14	12
Number of females	10	12
Number of smokers	0	0
Age, range (mean ± SD)	39–58 (47 ± 5)	37–54 (46 ± 5)

SRP, scaling and rootplaning + placebo tablet; SRP + P: scaling and rootplaning + probiotic tablet.

Table 2. Mean (±standard deviation) probing pocket depth (PPD) outcome measures, clinical attachment level (CAL), recession (REC), bleeding on probing (BoP) outcome measures at baseline, 12 and 24 weeks

| Variable | Time point | Treatment group | | | | p-Value | |
| | | SRP | | SRP + P | | | |
		Mean ± SD	Delta ± SD	Mean ± SD	Delta ± SD	For mean	For Delta
PPD (mm)							
Overall	Baseline	4.59 ± 0.52		4.50 ± 0.51		NS	
	12 weeks	3.26 ± 0.49*	−1.34 ± 0.33	3.15 ± 0.52*	−1.35 ± 0.30	NS	NS
	24 weeks	2.98 ± 0.47*	−1.62 ± 0.41	2.99 ± 0.47*	−1.52 ± 0.38	NS	NS
Moderate pockets	Baseline	4.82 ± 0.25		4.83 ± 0.24		NS	
	12 weeks	3.29 ± 0.40*	−1.54 ± 0.35	3.21 ± 0.45*	−1.62 ± 0.35	NS	NS
	24 weeks	3.01 ± 0.41*	−1.82 ± 0.42	3.05 ± 0.42*	−1.78 ± 0.38	NS	NS
Deep pockets	Baseline	7.18 ± 0.24		7.13 ± 0.20		NS	
	12 weeks	4.78 ± 0.79*	−2.41 ± 0.69	4.76 ± 0.90*	−2.37 ± 0.83	NS	NS
	24 weeks	3.76 ± 0.87*	−3.43 ± 0.84	3.93 ± 1.02*	−3.20 ± 0.99	NS	NS
CAL (mm)							
Overall	Baseline	5.36 ± 0.45		5.22 ± 0.41		NS	
	12 weeks	4.66 ± 0.45*	−0.70 ± 0.30	4.47 ± 0.39*	−0.76 ± 0.20	NS	NS
	24 weeks	4.60 ± 0.48*	−0.75 ± 0.37	4.51 ± 0.41*	−0.71 ± 0.28	NS	NS
Moderate pockets	Baseline	5.54 ± 0.24		5.53 ± 0.27		NS	
	12 weeks	4.62 ± 0.32*	−0.92 ± 0.22	4.50 ± 0.30*	−1.03 ± 0.21	NS	NS
	24 weeks	4.57 ± 0.38*	−0.98 ± 0.30	4.55 ± 0.35*	−0.98 ± 0.25	NS	NS
Deep pockets	Baseline	8.26 ± 0.62		8.09 ± 0.59		NS	
	12 weeks	6.87 ± 0.83*	−1.39 ± 0.76	6.77 ± 0.53*	−1.32 ± 0.68	NS	NS
	24 weeks	6.44 ± 0.63*	−1.82 ± 0.65	6.44 ± 0.40*	−1.65 ± 0.54	NS	NS
REC (mm)							
Overall	Baseline	0.76 ± 0.26		0.72 ± 0.30		NS	
	12 weeks	1.40 ± 0.44*	0.65 ± 0.26	1.31 ± 0.43*	0.59 ± 0.25	NS	NS
	24 weeks	1.62 ± 0.38*	0.87 ± 0.23	1.52 ± 0.45*	0.80 ± 0.32	NS	NS
BoP (%)							
Overall	Baseline	85.55 ± 7.29		87.44 ± 6.03		NS	
	12 weeks	28.31 ± 7.71*	−0.57 ± 0.09	27.74 ± 10.34*	−0.60 ± 0.12	NS	NS
	24 weeks	30.11 ± 10.36*	−0.48 ± 0.19	26.98 ± 9.34*	−0.47 ± 0.17	NS	NS

*Significant different from baseline.
SD, standard deviation; SRP, scaling and rootplaning + placebo tablet; SRP + P, scaling and rootplaning + probiotic tablet.
Significance of differences between groups: $p > 0.1$: not significant (NS), $p \leq 0.1$ to > 0.05: tendency, $p \leq 0.05$.

Table 3. Mean percentages of sites with plaque and gingival bleeding at baseline, 12 and 24 weeks

| Variable | Time point | Treatment group | | p-value |
		SRP Mean	SRP + P Mean	
% sites with supragingival plaque				
Overall	Baseline	99.7	99.0	NS
	4 weeks	55.5	54.0*	NS
	8 weeks	55.2	51.7*	NS
	12 weeks	60.5	57.9*	NS
	24 weeks	73.1	51.7*	**0.0024**
% sites with gingivitis				
Overall	Baseline	99.1	97.9	NS
	4 weeks	43.7*	45.5*	NS
	8 weeks	46.2*	39.6*	NS
	12 weeks	50.8*	42.6*	NS
	24 weeks	59.0*	44.0*	0.068

*Significant different from baseline.
SD, standard deviation; SRP, scaling and rootplaning + placebo tablet; SRP + P, scaling and rootplaning + probiotic tablet.
Significance of differences between groups: $p > 0.1$: not significant (NS), $p \leq 0.1$ to > 0.05: tendency, $p \leq 0.05$: significant (bold).

variable for the moderate (initial PPD between 4 and 6 mm) and deep (initial PPD ≥ 7 mm) pockets. To examine the clinical impact of this parameter, the "Risk for disease progression" was calculated also showing no significant difference between both groups.

For the secondary outcome measures (CAL, REC, BOP, PI, GI, need for additional surgery and microbiological data), in both groups significant improvements ($p < 0.05$) were noted at the 12- and the 24-week evaluation. However, no inter-group differences could be detected at any time point, except from less sites with plaque in the probiotic group at the 24-week evaluation and lower *P. intermedia* counts in saliva for the probiotic group after 12 weeks. Since no adverse events are reported, it can

Table 4. Mean (±standard deviation) for microbiological outcome measures in subgingival plaque

Variable	Time point	Mean log10 CFU/ml ± SD			Delta mean log10 CFU/ml		
		SRP	SRP + P	*p*-value	SRP	SRP + P	*p*-value
Fusobacterium nucleatum	Baseline	5.81 ± 0.62	5.81 ± 0.49	NS			
	4 weeks	3.14 ± 1.33*	2.81 ± 1.78*	NS	−2.67 ± 1.16	−2.99 ± 1.61	NS
	8 weeks	3.97 ± 1.21*	3.94 ± 1.42*	NS	−1.83 ± 0.89	−1.86 ± 1.33	NS
	12 weeks	4.64 ± 0.80*	4.65 ± 0.75*	NS	−1.17 ± 0.63	−1.16 ± 0.68	NS
	24 weeks	5.22 ± 0.66*	5.18 ± 0.65*	NS	−0.59 ± 0.47	−0.63 ± 0.51	NS
Porphyromonas gingivalis	Baseline	6.24 ± 1.21	6.16 ± 1.14	NS			
	4 weeks	2.07 ± 1.96*	1.88 ± 1.93*	NS	−4.16 ± 1.90	−2.99 ± 1.61	NS
	8 weeks	3.15 ± 1.99*	3.39 ± 1.69*	NS	−3.09 ± 1.61	−1.86 ± 1.33	NS
	12 weeks	4.07 ± 1.91*	4.91 ± 1.38*	NS	−2.17 ± 1.39	−1.16 ± 0.68	NS
	24 weeks	4.97 ± 1.73*	5.49 ± 1.34*	NS	−1.27 ± 1.10	−0.63 ± 0.51	NS
Prevotella intermedia	Baseline	4.17 ± 1.83	4.52 ± 1.59	NS			
	4 weeks	0.27 ± 0.91*	0.30 ± 1.02*	NS	−3.90 ± 1.86	−4.22 ± 1.73	NS
	8 weeks	0.86 ± 1.53*	1.24 ± 1.69*	NS	−3.32 ± 1.97	−3.28 ± 1.91	NS
	12 weeks	1.47 ± 1.85*	2.36 ± 2.02*	NS	−2.70 ± 2.02	−2.16 ± 1.69	NS
	24 weeks	2.74 ± 1.99	3.59 ± 1.88*	NS	−1.43 ± 1.49	−0.93 ± 1.38	NS
Tannerella forsythia	Baseline	5.36 ± 0.72	5.11 ± 1.39	NS			
	4 weeks	0.68 ± 1.38*	1.24 ± 1.90*	NS	−4.69 ± 1.18	−3.87 ± 1.93	NS
	8 weeks	2.03 ± 1.84*	2.08 ± 2.12*	NS	−3.33 ± 1.63	−3.03 ± 1.85	NS
	12 weeks	3.52 ± 1.58*	3.52 ± 2.07*	NS	−1.85 ± 1.50	−1.59 ± 1.56	NS
	24 weeks	4.60 ± 0.86*	4.42 ± 1.62*	NS	−0.76 ± 0.71	−0.70 ± 0.81	NS

*Significant different from baseline.
CFU, colony-forming units; SD, standard deviation; SRP, scaling and rootplaning + placebo tablet; SRP + P, scaling and rootplaning + probiotic tablet.
Significance of differences between groups: $p > 0.1$: not significant (NS), $p \leq 0.1$ to > 0.05: tendency, $p \leq 0.05$.

Table 5. Mean (±standard deviation) for microbiological outcome measures in supragingival plaque

Variable	Time point	Mean log10 CFU/mL ± SD			Delta mean log10 CFU/ml		
		SRP	SRP + P	*p*-value	SRP	SRP + P	*p*-value
Fusobacterium nucleatum	Baseline	5.34 ± 0.72	5.57 ± 0.57	NS			
	4 weeks	3.02 ± 1.53*	3.04 ± 1.49*	NS	−2.33 ± 1.33	−2.53 ± 1.50	NS
	8 weeks	3.98 ± 0.78*	4.27 ± 0.57*	NS	−1.36 ± 0.66	−1.30 ± 0.49	NS
	12 weeks	4.47 ± 0.80*	4.70 ± 0.56*	NS	−0.87 ± 0.48	−0.87 ± 0.41	NS
	24 weeks	4.83 ± 0.68*	5.13 ± 0.58*	NS	−0.51 ± 0.47	−0.44 ± 0.28	NS
Porphyromonas gingivalis	Baseline	5.63 ± 1.67	5.67 ± 1.19	NS			
	4 weeks	0.85 ± 1.36*	1.44 ± 1.66*	NS	−4.78 ± 1.80	−2.53 ± 1.50	NS
	8 weeks	2.30 ± 1.50*	2.50 ± 1.64*	NS	−3.33 ± 1.47	−1.30 ± 0.49	NS
	12 weeks	3.27 ± 1.27*	3.66 ± 1.52*	NS	−2.36 ± 1.36	−0.87 ± 0.41	NS
	24 weeks	4.34 ± 1.42*	4.84 ± 1.18*	NS	−1.30 ± 1.05	−0.44 ± 0.28	NS
Prevotella intermedia	Baseline	4.29 ± 2.54	3.77 ± 2.41	NS			
	4 weeks	0.00 ± 0.00	0.13 ± 0.63	NS	−4.29 ± 2.54	−3.64 ± 2.39	NS
	8 weeks	0.39 ± 1.08	0.65 ± 1.33	NS	−3.90 ± 2.35	−3.12 ± 2.39	NS
	12 weeks	0.97 ± 1.59	1.98 ± 2.00	NS	−3.32 ± 2.34	−1.79 ± 1.80	NS
	24 weeks	2.84 ± 2.27	3.00 ± 2.25	NS	−1.45 ± 1.72	−0.77 ± 1.00	NS
Tannerella forsythia	Baseline	4.63 ± 1.29	4.52 ± 1.31	NS			
	4 weeks	0.59 ± 1.18*	1.04 ± 1.57*	NS	−4.04 ± 1.34	−3.49 ± 1.55	**0.06**
	8 weeks	1.51 ± 1.88*	1.81 ± 1.81*	NS	−3.12 ± 1.51	−2.72 ± 1.60	NS
	12 weeks	2.56 ± 1.84*	2.81 ± 1.99*	NS	−2.07 ± 1.58	−1.72 ± 1.45	NS
	24 weeks	3.67 ± 1.62*	3.75 ± 1.66*	NS	−0.97 ± 0.95	−0.77 ± 0.98	NS

*Significant different from baseline.
CFU, colony-forming units; SD, standard deviation; SRP, scaling and rootplaning + placebo tablet; SRP + P: scaling and rootplaning + probiotic tablet.
Significance of differences between groups: $p > 0.1$: not significant (NS), $p \leq 0.1$ to > 0.05: tendency, $p \leq 0.05$: significant (bold).

be concluded that this product is safe for human use.

As far as we know, this is the first randomized placebo-controlled trial examining the effect of a streptococci containing probiotic as an adjunct to conventional non-surgical periodontal therapy (SRP). Intuitively, the use of certain streptococci to improve periodontal health seemed logical. Already in the 1980s, a negative correlation was found between viridans streptococci and certain periodontopathogens (Hillman & Socransky 1982, Haffajee et al. 1984, Hillman et al. 1985). *Streptococcus sanguinis* (at this moment called *S. sanguinis*) and

Table 6. Mean (± standard deviation) for microbiological outcome measures at the tongue

Variable	Time point	Mean log10 CFU/ml ± SD			Delta mean log10 CFU/ml		
		SRP	SRP + P	*p*-value	SRP	SRP + P	*p*-value
Fusobacterium nucleatum	Baseline	4.71 ± 1.11	4.70 ± 0.85	NS			
	4 weeks	0.73 ± 1.41*	0.72 ± 1.53*	NS	−3.98 ± 1.57	−3.98 ± 1.64	NS
	8 weeks	1.67 ± 1.73*	1.97 ± 1.75*	NS	−3.33 ± 1.77	−2.74 ± 1.55	NS
	12 weeks	2.91 ± 1.84*	2.95 ± 1.64*	NS	−1.84 ± 1.55	−1.75 ± 1.39	NS
	24 weeks	3.80 ± 1.38*	3.76 ± 0.91*	NS	−0.86 ± 1.19	−0.95 ± 0.74	NS
Porphyromonas gingivalis	Baseline	4.25 ± 1.36	4.51 ± 1.41	NS			
	4 weeks	1.41 ± 1.47*	1.84 ± 1.74*	NS	−2.84 ± 1.36	−3.98 ± 1.64	NS
	8 weeks	1.89 ± 1.70*	2.58 ± 1.75*	NS	−2.36 ± 1.32	−2.74 ± 1.55	NS
	12 weeks	2.67 ± 1.79*	3.31 ± 1.39*	NS	−1.58 ± 1.26	−1.75 ± 1.39	NS
	24 weeks	3.60 ± 1.32*	3.71 ± 1.50*	NS	−0.64 ± 0.59	−0.95 ± 0.74	NS
Prevotella intermedia	Baseline	3.16 ± 2.39	3.78 ± 1.72	NS			
	4 weeks	0.10 ± 0.47*	0.31 ± 1.12*	NS	−3.06 ± 2.35	−3.48 ± 1.69	NS
	8 weeks	0.78 ± 1.42*	1.05 ± 1.78*	NS	−2.37 ± 2.02	−2.74 ± 1.74	NS
	12 weeks	1.79 ± 2.23*	1.47 ± 2.02*	NS	−1.37 ± 1.68	−2.32 ± 1.85	NS
	24 weeks	2.07 ± 2.37*	2.87 ± 1.90*	NS	−1.09 ± 1.56	−0.92 ± 1.08	NS
Tannerella forsythia	Baseline	3.56 ± 0.67	3.26 ± 1.59	NS			
	4 weeks	0.70 ± 1.25*	0.35 ± 0.96*	NS	−2.85 ± 1.24	−2.91 ± 1.65	NS
	8 weeks	1.11 ± 1.48*	1.34 ± 1.64*	NS	−2.45 ± 1.39	−1.92 ± 1.62	NS
	12 weeks	1.83 ± 1.61*	2.18 ± 1.64*	NS	−1.73 ± 1.17	−1.08 ± 1.13	NS
	24 weeks	2.63 ± 1.47*	2.92 ± 1.46*	NS	−0.92 ± 1.07	−0.34 ± 0.35	NS

*Significant different from baseline.
CFU, colony-forming units; SD, standard deviation; SRP, scaling and rootplaning + placebo tablet; SRP + P, scaling and rootplaning + probiotic tablet.
Significance of differences between groups: *p* > 0.1: not significant (NS), *p* ≤ 0.1 to > 0.05: tendency, *p* ≤ 0.05.

Table 7. Mean (±standard deviation) for microbiological outcome measures in saliva

Variable	Time point	Mean log10 CFU/ml ± SD			Delta mean log10 CFU/ml		
		SRP	SRP + P	*p*-Value	SRP	SRP + P	*p*-Value
Fusobacterium nucleatum	Baseline	5.30 ± 1.33	5.63 ± 0.67	NS			
	4 weeks	2.40 ± 1.68*	2.47 ± 1.77*	NS	−2.90 ± 1.59	−3.15 ± 1.68	NS
	8 weeks	3.44 ± 1.59*	3.66 ± 1.70*	NS	−1.86 ± 1.22	−1.97 ± 1.41	NS
	12 weeks	4.15 ± 1.33*	4.33 ± 1.32*	NS	−1.15 ± 0.87	−1.30 ± 1.08	NS
	24 weeks	4.60 ± 1.34*	4.93 ± 0.97*	NS	−0.70 ± 0.77	−0.69 ± 0.62	NS
Porphyromonas gingivalis	Baseline	5.06 ± 1.04	5.17 ± 1.25	NS			
	4 weeks	1.66 ± 1.77*	2.49 ± 1.70*	NS	−3.41 ± 1.53	−3.15 ± 1.68	NS
	8 weeks	2.58 ± 1.50*	3.27 ± 1.47*	NS	−2.48 ± 1.27	−1.97 ± 1.41	NS
	12 weeks	3.37 ± 1.23*	3.92 ± 1.27*	NS	−1.69 ± 1.10	−1.30 ± 1.08	NS
	24 weeks	4.01 ± 0.86*	4.40 ± 1.44*	NS	−1.05 ± 0.82	−0.69 ± 0.62	NS
Prevotella intermedia	Baseline	3.37 ± 2.00	3.55 ± 1.91	NS			
	4 weeks	0.34 ± 0.91*	0.62 ± 1.44*	NS	−3.03 ± 1.93	−2.93 ± 1.74	NS
	8 weeks	0.96 ± 1.28*	1.41 ± 1.84*	NS	−2.40 ± 1.67	−2.14 ± 1.68	NS
	12 weeks	1.21 ± 1.49*	2.01 ± 2.00*	NS	−2.16 ± 1.51	−1.54 ± 1.36	**0.02**
	24 weeks	2.41 ± 1.60*	2.78 ± 2.01*	NS	−0.96 ± 1.14	−0.77 ± 1.01	NS
Tannerella forsythia	Baseline	1.18 ± 0.76	4.91 ± 0.94	NS			
	4 weeks	2.21 ± 1.77*	1.90 ± 1.72	NS	−3.69 ± 1.55	−3.02 ± 1.45	NS
	8 weeks	3.18 ± 1.87*	2.82 ± 1.83*	NS	−2.66 ± 1.39	−2.10 ± 1.23	NS
	12 weeks	4.17 ± 1.65*	3.51 ± 1.96*	NS	−1.69 ± 1.37	−1.41 ± 1.29	NS
	24 weeks	1.18 ± 1.27*	4.08 ± 1.52*	NS	−0.70 ± 1.04	−0.84 ± 0.98	NS

*Significant different from baseline.
CFU, colony-forming units; SD, standard deviation; SRP, scaling and rootplaning + placebo tablet; SRP + P, scaling and rootplaning + probiotic tablet.
Significance of differences between groups: *p* > 0.1: not significant (NS), *p* ≤ 0.1 to > 0.05: tendency, *p* ≤ 0.05: significant (bold).

S. uberis are able to produce hydrogen peroxide, which inhibits the growth of certain periodontopathogens (e.g. *A. actinomycetemcomitans*) (Hillman et al. 1985). More recently, it was demonstrated that streptococci are correlated with a healthy oral condition (Socransky et al. 1998, Haffajee et al. 2008, Loozen et al. 2014). In addition, the subgingival application of streptococci in a beagle dog model after SRP retard the re-colonization of periodontopathogens and improve the clinical outcome (Teughels et al.

2007, Nackaerts et al. 2008). Moreover, some streptococci are known to attenuate the inflammatory response elicited by pathogenic bacteria (Sliepen et al. 2009, Kaci et al. 2011, 2014, Zhang & Rudney 2011). However, this randomized placebo-controlled trial could not demonstrate clinical, nor microbiological benefits for the use of the investigated streptococci containing probiotic in humans. These microbiological findings are in accordance with Zahradnik et al. (2009) who investigated a Streptococci mouthwash.

The clinical and microbiological data are, however, in contrast with the available RCT's on *Lactobacillus reuteri* probiotics as an adjunct to SRP. Vivekananda (2010) demonstrated significant more PPD reduction, clinical attachment gain, better plaque, gingival and gingival bleeding indexes in patients using a *L. reuteri* probiotic in addition to SRP compared to SRP alone. In addition, Teughels et al. (2013) showed in a similar patient population that there were significant better results for pocket depth reduction and clinical attachment gain in moderate and deep pockets in the group receiving *L. reuteri* lozenges after SRP compared with the group receiving a placebo. Recently Tekçe et al. (2015) showed significantly better PI, GI, BOP and PPD up to one year after receiving a *L. reuteri* lozenges for 3 weeks after SRP *versus* the use of a placebo. A significantly greater reduction of obligate anaerobe counts was shown up to 180 days comparing the probiotic with the placebo group. İnce et al. (2015) showed also significant better PI, GI, BOP and PPD in favour of the probiotic group up to 1-year. In addition, matrix metalloprotein-8 and tissue inhibitor of metalloproteinases-1 levels were decreased in gingival crevicular fluid up to 180 days after a 3 weeks use of a probiotic compared with a placebo (İnce et al. 2015).

The lack of previous RCT's on this streptococci containing probiotic product implicated that there were no data to support an a priori power analysis. Therefore, the power analysis was based on Vivekananda and co-workers, which used a similar study set-up although the used probiotic product was different (Vivekananda et al. 2010). Although this analysis showed that 10 patients per group would be sufficient, this number was raised to 24. The latter decision was supported by Zahradnik et al. (2009) who calculated that to find statistically significant microbiological differences, 24 patients would be needed. Despite these numbers, no statistically significant differences could be found between the probiotic and placebo group. Based on the data of the current trial, a post hoc power analysis was conducted showing that eight times more patients are needed to show a statistically significant difference for PPD at 12 weeks.

Another drawback of this study was that no attempt was made to check whether the probiotic bacteria actually colonized the oral cavity. The reason for not doing this was technical in nature. Currently, there are no microbiological techniques available which have enough sensitivity and specificity to detect these bacterial strains in frozen samples. Moreover, strain specific detection would be mandatory since *S. oralis* and *S. uberis* are abundant species in the oral cavity. However, their detection would not have made any difference in the final clinical conclusion of this study.

Overall, when comparing the data of this study with the data Teughels et al. (2013), which had an almost identical set-up, it is clear that the clinical and microbiological effects of probiotic supplements are species specific. Most likely, this specificity can be extended to strain, dosage and vehicle specific effects. At the end, this means that, when evaluating the clinical and microbiological effects of probiotic supplements, one is currently actually looking at product specific effects rather than merely the effects of (species specific) probiotics (e.g. streptococci containing probiotics) in general. Future systematic reviews and meta-analysis should take this into account. In addition, this also means that this study did not prove or disprove the hypothesis that oral streptococci might be more potent probiotics for instance lactobacilli (Teughels et al. 2011, Tonetti & Chapple 2011). The outcome of this study can only be interpreted in relation to the described study parameters. Other strains, other dosages, other modes of application could still result in different clinical and microbiological results.

In conclusion, this study showed almost no effect of the usage of a probiotic tablet containing *S. oralis* KJ3, *S. uberis* KJ2 and *S. rattus* JH145 as a supplement to SRP on neither clinical nor microbiological parameters. Only at the 24-week evaluation the sites with plaque detected are significantly lower in the probiotic group than in de control group and after 12 week lower *P. intermedia* counts in saliva were detected in the probiotic group. Therefore, at this moment, there is no evidence for the use of this probiotic tablet in the daily clinical practice for adult periodontitis patients. Since this is the only available RCT describing the usage of this product, further research is needed to investigate its influence on periodontal health.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart.
Table S1. Number of patients according to risk for disease progression (Lang & Tonetti 2003) (%) at 12 and 24 weeks.
Table S2. Need for surgery outcome measures at baseline, 12 and 24 weeks.

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Clinical Relevance

Scientific rationale for the study: Streptococci are omnipresent in the oral cavity and re-colonize the periodontal pockets soon after SRP. However, the adjunctive use of streptococci as probiotics to SRP has not yet been described.

Principal findings: This study did not find clinical nor microbiological significant differences between periodontitis patients receiving a placebo or a probiotic tablet after SRP.

Practical implications: To date, there is no evidence to use a streptococci containing probiotic tablet in addition to SRP in adult periodontitis patients. Based on the scientific literature, the effect of probiotics seems to be product related.