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Probiotic effects of orally administered *Lactobacillus salivarius* WB21-containing tablets on periodontopathic bacteria: a double-blinded, placebo-controlled, randomized clinical trial

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Abstract

Aim: This study was designed to evaluate whether the oral administration of lactobacilli could change the bacterial population in supra/subgingival plaque.

Material and Methods: Sixty-six healthy volunteers without severe periodontitis were randomized into two groups to receive lactobacilli or placebo for 8 weeks (8W): the test group ($n = 34$) received 2.01×10^9 CFU/day of *Lactobacillus salivarius* WB21 and xylitol in tablets; the control group ($n = 32$) received placebo with xylitol. Supra/subgingival plaque samples were collected at the baseline and after 4 weeks (4W) and 8W. The bacterial amounts in plaque samples were analysed by quantitative real-time polymerase chain reaction.

Results: The numerical sum of five selected periodontopathic bacteria in the test group was decreased significantly in subgingival plaque at 4W [odds ratio (OR) = 3.13, 95% confidence intervals (CI) = 1.28–7.65, $p = 0.012$]. Multivariate analysis showed that significantly higher odds were obtained for the reduction of *Tannerella forsythia* in subgingival plaque of the test group at both 4W (OR = 6.69, 95% CI = 2.51–17.9, $p < 0.001$) and 8W (OR = 3.67, 95% CI = 1.45–9.26, $p = 0.006$).

Conclusion: Oral administration of probiotic lactobacilli reduced the numerical sum of five selected periodontopathic bacteria and could contribute to the beneficial effects on periodontal conditions.

Key words: dental plaque; double-blind method; *Lactobacillus salivarius*; microbiology; periodontitis; placebos; probiotics; randomized-controlled trial

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Conflict of interest and sources of funding statement

None of the other authors declare any potential conflicts of interests.
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Probiotics are live microorganisms, which, when administered in an adequate amount, confer a health benefit on the host (FAO/WHO 2001). The effect of probiotic therapy has been studied extensively for a variety of systemic indications and medical disorders. For example, the potential benefits of probiotics have been studied in gastrointestinal disorders (Naidu et al. 1999, Vanderhoof et al. 1999), gynaecology (Hilton et al. 1995, McLean & Rosenstein 2000, Reid et al. 2003), and atopic eczema (Kalliomaki et al. 2001). Probiotic bacteria can provide health benefits to the host by (1) providing nutrients and cofactors to the host, (2) competing directly with pathogens, (3) interacting with pathogen virulence factors, and (4) stimulating host immune responses (Saier & Mansour 2005). Thus, therapeutic targets for probiotics are now

widely distributed among infectious and non-infectious diseases.

Dental caries and periodontitis are the most common infectious diseases in humans (Çaglar et al. 2005). Recently, there has been increasing interest in probiotic control against these oral infections, and a number of clinical trials have been conducted to elucidate the possible impact on oral health. Twetman & Stecksén-Blicks (2008) reviewed these trials and concluded that probiotic intervention in childhood may hamper the presence of mutans streptococci in saliva, possibly reducing the risk of dental caries. However, only limited information is available regarding the effect of probiotics on periodontal health and clinical conditions. Krasse et al. (2006) first showed decreased gingival bleeding and reduced gingivitis by the administration of probiotic *Lactobacillus reuteri*. It was reported recently that oral administration of probiotic *Lactobacillus salivarius* WB21 successfully improved the periodontal condition of healthy volunteers, especially for smoker subjects, but not for non-smokers (never/former smokers), in a double blind, placebo-controlled, randomized clinical trial (Shimauchi et al. 2008).

Probiotic intervention in the oral cavity is considered to exert its effect by microbiological interference with preexisting microbiota and supposed pathogens. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are widely regarded as major periodontal pathogens (Socransky et al. 1998). *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* have also been found in subgingival lesions in periodontitis patients (Ashimoto et al. 1996, Zambon 1996, Loomer 2004). Regarding the probiotic effects on periodontal pathogens, Grudianov et al. (2002) reported that the use of probiotic tablets was effective for the normalization of microbiota in periodontitis and gingivitis patients as compared with a control group. In Japan, Ishikawa et al. (2003) and Matsuoka et al. (2006) reported that the administration of *L. salivarius* TI2711 (LS1) to healthy subjects neutralized the pH of saliva and considerably decreased the numbers of black-pigmented anaerobic rods in saliva. Taking these previous reports together, probiotics may exert beneficial effects not only on the clinical condition of the oral cavity but also on the microbiota of periodontal tissues. Nevertheless, the effects of probiotics on periodontopathic bacteria in the oral cavity have not been fully elucidated.

Lactobacilli and bifidobacteria are the bacteria that are used most frequently as probiotics. Lactobacilli are indigenous bacteria colonizing the oral cavity and digestive tract and have been shown to play key roles in the prevention and treatment of gastrointestinal disorders (Reid et al. 1990). Lactobacilli constitute approximately 1% of cultivable oral microbiota (Marsh & Martin 1999). *L. salivarius* strain WB21 was selected as a probiotic, which was bred selectively from *L. salivarius* WB1004 (Aiba et al. 1998) by a low-pH treatment (pH 1.2) to exert a probiotic effect in the gastrointestinal tract. This strain is resistant to gastric acid and nominated as a candidate probiotic bacteria. Lactic acid bacteria can produce different antimicrobial components including organic acids, hydrogen peroxide, low-molecular-weight antimicrobial substances, bacteriocins, and adhesion inhibitors (Silva et al. 1987). These antimicrobial components should also be effective in the inhibition of periodontopathic bacteria.

The aim of this study was to evaluate whether the oral administration of *L. salivarius* WB21 could reduce the levels of periodontopathic bacteria in the oral cavity. For this purpose, a double blind, randomized, placebo-controlled clinical trial was conducted in healthy volunteers without severe periodontitis.

Material and Methods

Subjects and study design

This study was approved by the Research Ethics Committee of Tohoku University Graduate School of Dentistry. The characteristics of the study subjects and the methods used in the intervention study were detailed previously (Shimauchi et al. 2008). The important aspects are described briefly below.

Briefly, 71 healthy volunteers were recruited from company workers (Wakamoto Pharmaceutical Co., Tokyo, Japan) and each provided written informed consent for this study. The recruits were outwardly healthy and further confirmed to meet the following criteria: (1) not currently visiting their dentists for treatment; (2) not using probiotic supplements; (3) free of adverse reactions to lactose or fermented milk products; and (4) not taking antibiotics within the last month. Clinical parameters, including probing pocket depth (PPD), gingival index (GI; Löe & Silness 1963), bleeding on probing (BOP; Ainamo & Bay 1975), and plaque index (PlI; Silness & Löe 1964), were obtained from the Ramfjord's six teeth (Ramfjord 1959) at baseline (BL) and severe periodontitis subjects were excluded from the study. The exclusion criteria for those applicants were (1) PPD $\geqslant$ 6 mm for at least one periodontal pocket of the examined teeth and (2) the presence of teeth with excess mobility and/or abscess formation. Four subjects were excluded and 67 eligible subjects (58 males and nine females; mean age 44.9 ± 8.3 years, range 32–61 years) were allocated randomly to the test and control groups, according to gender, age, and smoking status (Fig. 1) by one of the authors (H. H.), who had little involvement with any assessments, using a randomization table. H. H. was independent throughout the study and held the randomization code until the start of data analysis. The randomization code was broken when all microbiological and clinical data were gathered after the 8-week (8W) intervention period.

A randomized, double-blinded, placebo-controlled study design with two parallel groups was used in this study. The subjects were randomized into two groups to receive test (WB21 group) and control (placebo group) treatments after the BL examination. There were no significant differences between the WB21 and placebo groups randomized according to gender, age, smoking habits, and clinical features at BL (Table 1). Tablets containing *L. salivarius* WB21 (6.7×10^8 CFU/tab) and xylitol (280 mg/tab) (WB21; WAKAMATE D®, Wakamoto Pharmaceutical Co.) were used for the WB21 group, and only xylitol was used for the placebo group. Each subject was instructed to place one tablet in the mouth and allow it to dissolve without chewing, three times a day for 8W. Participants in both groups were instructed not to change their oral hygiene regimens and not to take other probiotic products throughout the test period. Neither professional prophylaxis nor tooth brushing instruction was performed during or before the experimental period.

Collection of samples

Both supragingival and subgingival plaque samples were taken from the mesial

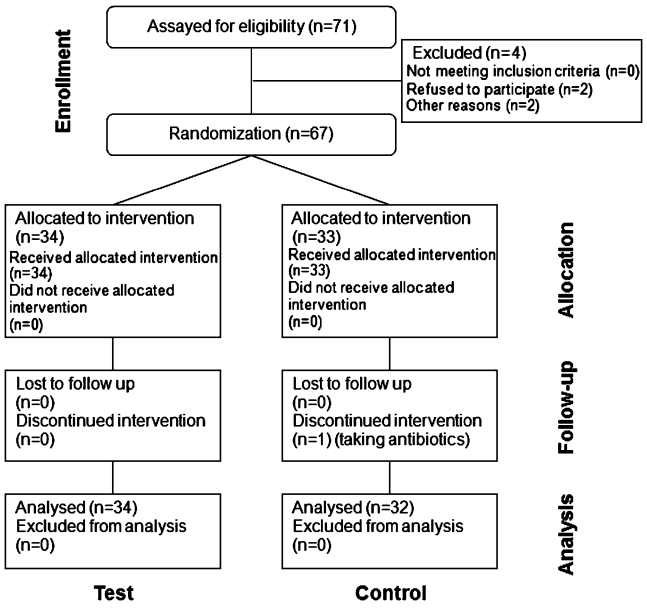

Fig. 1. Flowchart of the subjects throughout the study.

Table 1. Clinical characteristics of the subjects

Characteristics	WB21 group	Placebo group	*p*-value
Subjects	34	32	
Gender (male:female)	29:5	28:4	0.79
Mean age (years)	45.0 ± 8.3	44.8 ± 8.4	0.87
Smoking status			0.98
Current	8	7	
Former	12	11	
Never	14	14	
Clinical parameters			
PPD (mm)	2.5 ± 0.1	2.4 ± 0.2	0.06
GI	0.8 ± 0.1	0.7 ± 0.1	0.11
BOP (%)	19.2 ± 2.4	13.9 ± 2.5	0.07
PII	0.7 ± 0.1	0.6 ± 0.1	0.38

Number or mean ± standard error (SE).
Student's *t*-test was used if the variable was continuous with a normal distribution and Mann–Whitney's *U*-test if the variable was continuous but with a skewed distribution. For categorical variables, χ^2 test or Fisher's Exact test were performed.
BOP, bleeding on probing; GI, gingival index; PlI, plaque index; PPD, probing pocket depth.

sites of the Ramfjord's 6 teeth at BL and after 4 weeks (4W) and 8W. Supragingival plaque samples were taken using sterile toothpicks from the mesial sites of the target teeth and gathered in a 1.5-ml sterile tube. For subgingival plaque sampling, the teeth were isolated with cotton rolls, and then supragingival plaque was removed with sterile cotton pellets. Then, a sterile paper point was inserted into the same site for probing. After 30 s, the points were removed and immersed into a tube containing 0.5 ml of sterile distilled water and then mixed using a vortex. All samples were stored at −20°C before extraction of genomic DNA.

Microbiological examination

Bacterial DNA was extracted from plaque as described previously (Sakamoto et al. 2001). In brief, lysozyme (final concentration 5 mg/ml) and *N*-acetylmuramidase (final concentration 0.1 mg/ml) were added to each suspension. After incubation at 37°C for 1 h, proteinase K and sodium dodecyl sulphate were added to a final concentration of 2 mg/ml and 1% (w/v), respectively. The mixture was incubated at 50°C for 30 min. Nucleic acids were released by three cycles of freezing in a −80°C freezer, followed by thawing in a 65°C water bath. The mixture was then extracted with equal volumes of phenol (saturated with 10 mM Tris-HCl, pH 8.0) and phenol–chloroform–isoamyl alcohol (25:24:1). Bulk nucleic acids were precipitated from the solution using isopropyl alcohol, followed by centrifugation (9510 *g*) for 30 min. The DNA precipitate was washed with 70% ethanol and resuspended in 100 μl of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Quantitative real-time polymerase chain reaction (PCR) amplifications were performed to detect five species of selected periodontopathic bacteria: *A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *T. denticola*, and *T. forsythia*. The sequences of the primers and the strains used for the standard curves are listed in Table 2. The PCR reaction was performed in a 50 μl final volume containing 5 μl template DNA, 25 μl SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA), and 0.5 μl of each primer, and amplified using an ABI PRISM 7300 Real-Time PCR System (Applied Biosystems) programmed for an initial denaturation step at 95°C for 10 min., followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. each. Fluorescent products were detected during the annealing–extension step. The bacterial cell numbers per sample were calculated using the standard curve made from each tested bacterial cells as reported previously (Sakamoto et al. 2001)

Statistical analysis

Demographic and BL characteristics were compared between the groups using Student's *t*-test for continuous variables with a normal distribution, Mann–Whitney's *U*-test for variables with a non-normal distribution, and χ^2 or Fisher's exact tests for categorical variables.

The number of bacteria was calculated for each plaque sample from the threshold cycle values using the constructed standard curves. As the detection limit for the bacterial count was $\leqslant 100$ using the present PCR system, the lowest value was given as 100 for the count of samples under the measurable limit. Both total bacterial counts and the sum of five periodontotal pathogens in plaque samples were continuous, but skewed and distributed non-normally. A proportional odds logistic regression model (McCullagh 1980) was used to compare the microbiologi-

Table 2. Target bacteria and the primers used in this study

Species	Sequence (5′–3′)	Strains for standard curve	References
Aggregatibacter actinomycetemcomitans	CTTACCTACTCTTGACATCCGAA ATGCAGCACCTGTCTCAAAGC	*A. actinomycetemcomitans* JCM2434	Maeda et al. (2003)
Prevotella intermedia	AATACCCGATGTTGTCCACA TTAGCCGGTCCTTATTCGAA	*P. intermedia* ATCC25611	Maeda et al. (2003)
Porphyromonas gingivalis	CTTGACTTCAGTGGCGGCAG AGGGAAGACGGTTTTCACCA	*P. gingivalis* JCM8525	Maeda et al. (2003)
Treponema denticola	TAATACCGAATGTGCTCATTTACAT TCAAAGAAGCATTCCCTCTTCTTCTTA	*T. denticola* JCM5225	Sakamoto et al. (2001)
Tanerella forsythia	ATCCTGGCTCAGGATGAACG TACGCATACCCATCCGCAA	*T. forsythia* ATCC43037	Suzuki et al. (2004)
Universal primer	GTGCTGCACGGCTGTCGTCA ACGTCATCCACACCTTCCTC	*P. gingivalis* JCM8525	Maeda et al. (2003)

Table 3. Microbiological characteristics of the subjects

	Supragingival plaque			Subgingival plaque		
	WB21 group	placebo group	*p*-value	WB21 group	placebo group	*p*-value
Aggregatibacter actinomycetemcomitans	2.99 ± 0.21	3.52 ± 0.23	0.10	3.07 ± 0.23	3.40 ± 0.29	0.45
Prevotella intermedia	3.59 ± 0.29	3.96 ± 0.30	0.38	2.71 ± 0.19	2.79 ± 0.20	0.73
Porphyromonas gingivalis	2.98 ± 0.32	3.05 ± 0.32	0.82	2.99 ± 0.32	2.85 ± 0.28	0.95
Treponema denticola	2.98 ± 0.23	3.14 ± 0.22	0.42	3.01 ± 0.23	2.76 ± 0.23	0.46
Tanerella forsythia	2.89 ± 0.21	3.16 ± 0.19	0.29	3.11 ± 0.22	3.58 ± 0.13	0.25
Sum of five periodontopathic bacteria	4.57 ± 0.26	5.02 ± 0.22	0.14	4.54 ± 0.26	4.77 ± 0.23	0.41
Total	8.86 ± 0.10	8.96 ± 0.12	0.44	8.23 ± 0.12	8.23 ± 0.12	0.86

Mean bacterial number ($\log_{10}$) ± standard error (SE).
p-values are according to Mann–Whitney's *U*-test.

cal effects of intervention between WB21 and placebo groups. This is a model not only for ordinal categorical outcome variables but also for skewed continuous outcome variables using ranks of data (Ely et al. 2007). Under the proportional odds model, the odds ratio (OR) is an effective one-parameter representation of a distributional shift between the outcome distributions of test groups. The null hypothesis for the model used in this study was that intake of *L. salivarius* WB21 was not more effective than placebo in decreasing bacterial counts. Therefore, the OR for reducing five selected periodontopathic bacteria from BL value at 4W and 8W was calculated in this model.

To assess the weight and significance of the change in each periodontopathic bacteria selected in the bacterial counts of plaque samples, a multivariate logistic regression model was generated including these changes as covariates, and used to provide estimate OR for the total change. To limit the chance of falsely rejecting the null hypothesis (no association) as a result of multiple testing, Bonferroni correction was performed by setting α at 0.01 (two-sided) instead of the usual 0.05 for all models. The results from proportional odds logistic regression analyses were presented with 95% confidence intervals (CI) for the OR. SPSS (SPSS Inc., Chicago, IL, USA) version 15.0 for Windows® was used for the data analysis.

Results

Compliance and adverse effects

The experiment was started in August 2005 and finished in October 2005. As reported previously (Shimauchi et al. 2008), one subject was lost in the control group during the 8W follow-up period. Sixty-six subjects, 34 in the test group and 32 in the placebo group, were analysed, and no adverse events were observed. The withdrawn subject took antibiotics after the 4W examination as prescribed by a physician because of respiratory infection and also stopped taking the tablets. However, this was judged not to be an adverse reaction related to this study.

Microbiological findings

The clinical characteristics of the WB21 and placebo groups at BL are summarized in Table 1. There were no significant differences at BL in any clinical parameter (PPD, GI, BOP, and PlI). The microbiological characteristics of supragingival and subgingival plaque samples from both groups are also presented in Table 3. No significant differences between the groups were detected in terms of the total bacterial number or the number of any of five selected species of periodontopathic bacteria (*A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *T. denticola*, and *T. forsythia*) in supragingival and subgingival plaque.

Figure 2 shows the changes in the numbers of total bacteria of supra/subgingival plaques of both groups during the intervention period. No between- or intra-group changes were observed for the number of total bacteria of both plaque samples by the 8W intervention. Changes in the sum of five selected species of periodontopathic in supragingival and subgingival plaque are displayed in Fig. 3. The sum of five periodontal pathogens in the subgingival plaque tended to decrease gradually in both groups. The difference between the groups was significant for subgingival plaque samples at 4W with estimated

OR = 3.13 (95% CI = 1.28–7.65, $p = 0.012$) for the WB21 group. The representation of changes in individual bacterial number during the 8W intervention is displayed in Figs 4 and 5. In supragingival (Fig. 4) and subgingival (Fig. 5) plaque samples, there was no significant difference between the WB21 and the placebo groups in the direct count of any specific periodontopathic bacteria. However, as shown in Fig. 3, the sum of five bacteria was significantly lower in the WB21 group at 4W, and tended to be lower up to 8W as compared with the placebo group. A multivariate model was used after adjusting for bacterial counts at BL, PlI, and smoking status, and only *T. forsythia* levels were significantly different between the groups (Table 4). The OR for reduction of *T. forsythia* in the WB21 group significantly increased at 4W and 8W as compared with the placebo group (4W: OR = 6.69; 95% CI = 2.51–17.9; $p < 0.001$, and 8W: OR = 3.67; 95% CI = 1.45–9.26; $p = 0.006$, respectively).

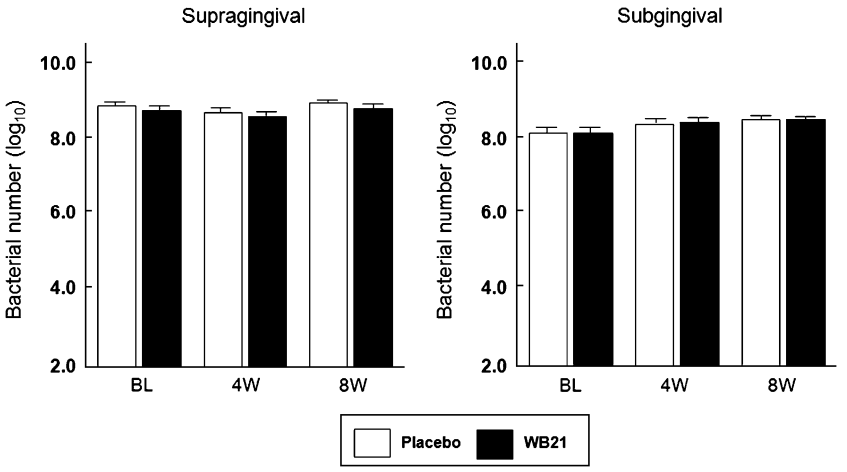

Fig. 2. Number of total bacteria in supragingival and subgingival plaque. Error bars indicate standard error.

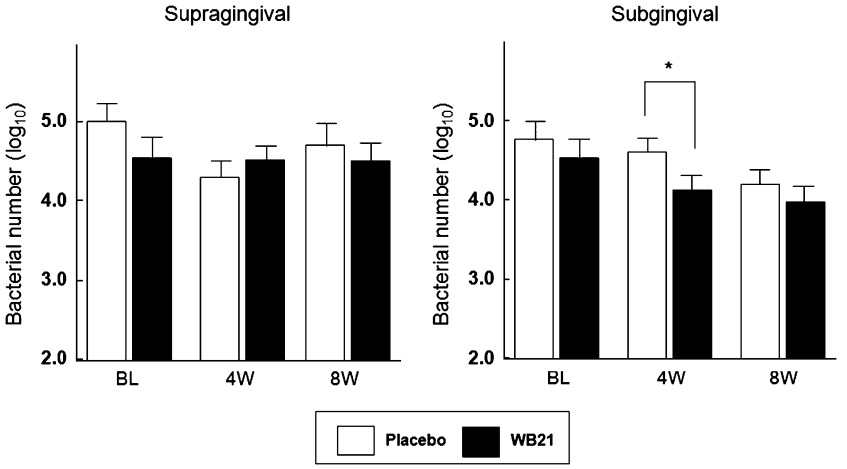

Fig. 3. Numerical sum of five selected periodontopathic bacteria in supragingival and subgingival plaque. Error bars indicate standard error. $^{*}p < 0.05$ significantly different by proportional odds logistic regression adjusting for bacterial count at baseline, plaque index, and smoking status.

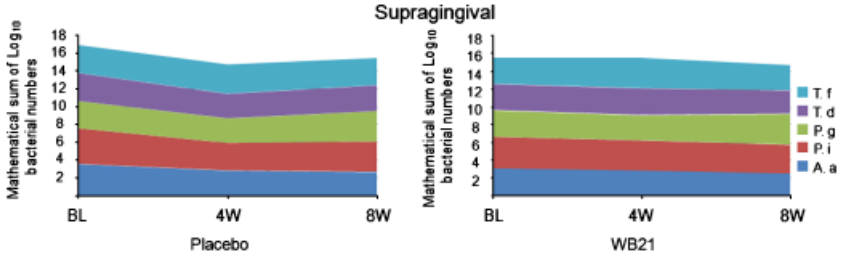

Fig. 4. Changes in the mean number of selected five periodontopathic bacteria during 8 weeks (8W) of intervention of supragingival plaque samples.

Discussion

The trial compared the effectiveness of orally administered *L. salivarius* WB21 on the microbiota of supra/subgingival plaque, especially on the fate of selected periodontopathic bacteria including *A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *T. denticola*, and *T. forsythia*. The present results showed a suppressive effect on the numerical sum of the counts of these periodontopathic bacteria in the subgingival plaque at after 4W of probiotic intervention. A significant reduction in *T. forsythia* at 4W and 8W was found in the subgingival plaque of the test group as compared with that of the placebo group. We previously reported that the impact of probiotic intervention with *L. salivarius* WB21 on clinical conditions of the same subjects enrolled in this RCT (Shimauchi et al. 2008). Briefly, significantly greater reductions of PlI and PPD were found at 4W and 8W for current smokers in the WB21 group as compared with those in the placebo group, although all clinical indices improved for both groups. Taken together, oral probiotics with *L. salivarius* WB21 could be a useful clinical tool for the treatment and prevention of peridodontal diseases by interfering with periodontal microbiota, especially by suppressing proposed pathogens.

Strains of *Lactobacillus* are known as major probiotic organisms in conjunction with *Bifidobacterium*, and the antagonistic ability of these strains against common microbial pathogens in the gastrointestinal tract has been well established. Lactobacilli exert an antimicrobial activity in the gastrointestinal tract or urovaginal environment by producing antimicrobial substances and/or stimulating mucosal immunity (Servin 2004). Hydrogen peroxide, metabolites such as lactic acid, biosurfactants, and small antimicrobial pep-

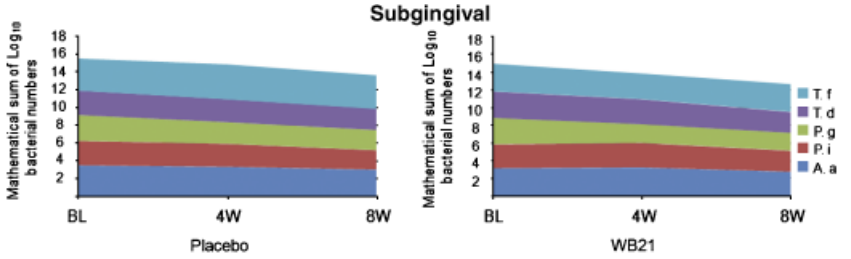

Fig. 5. Changes in the mean number of selected five periodontopathic bacteria during 8 weeks (8W) of intervention of subgingival plaque samples.

Table 4. Multivariable analysis of an improvement of the numbers of each species in subgingival plaque between the WB21 and the placebo groups

Bacteria	4W			8W		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Aggregatibacter actinomycetemcomitans	1.23	0.51–3.01	0.65	1.86	0.65–5.29	0.25
Prevotella intermedia	0.75	0.24–2.35	0.62	0.32	0.06–1.85	0.20
Porphyromonas gingivalis	8.00	0.72–88.6	0.09	6.66	0.39–113	0.19
Treponema denticola	0.61	0.21–1.74	0.35	2.04	0.52–8.05	0.31
Tanerella forsythia	6.69	2.51–17.9	<0.001*	3.67	1.45–9.26	0.006*

*$p<0.01$ significantly different by proportional odds logistic regression adjusting for bacterial count at baseline, PII, and smoking status.
CI, confidence interval; OR, odds ratio; 4W, 4 weeks; 8W, 8 weeks.

tides as bacteriocins are included in the antimicrobial molecules secreted from *Lactobacillus* spp. These bacteria have also been shown to activate immunocompetent cells to secrete both inflammatory and anti-inflammatory cytokines, resulting in modulation of the mucosal immune system. Presumably, probiotics may exert their beneficial effect in the oral cavity by both direct interactions with microorganisms in dental plaque and indirect actions such as modulation of the innate/acquired immune systems (Meurman 2005). These putative mechanisms could be the same in the oral cavity as they are in other parts, such as the gastrointestinal tract. However, because of the lack of probiotic research in odontology, these effects remain unclear.

In this study, *L. salivarius* WB21-derived probiotics significantly reduced the sum of five selected periodontal pathogens in the subgingival plaque at 4W as compared with the placebo group (Fig. 3). The precise mechanism for this inhibition by *L. salivarius* WB21 is not well understood. Probiotics with *L. salivarius* have been reported to reduce the number of *P. gingivalis* in saliva and subgingival plaque (Ishikawa et al. 2003, Matsuoka et al. 2006). Preliminary experiments also found that *L. salivarius* WB21 inhibited the growth of *P. gingivalis* and *P. intermedia* in vitro (data not shown), suggesting a direct interference with these bacteria. Very recently, Kõll et al. (2008) reported the antimicrobial effects of oral lactobacilli including *L. salivarius* on the growth of oral pathogenic bacteria. The majority of lactobacilli suppressed the growth of *A. actinomycetemcomitans, P. gingivalis, P. intermedia*, and *S. mutans*. It has been reported that the acidic environment inhibited the growth of these bacteria (Takahashi & Schachtele 1990, Takahashi et al. 1997). Thus, the lactic acid produced by lactobacilli is a plausible explanation for the inhibitory interaction with these organisms.

However, no reports are available regarding direct microbial interactions between lactobacilli and *T. forsythia*, which showed a significant reduction in the subgingival microbiota as compared with that of the placebo group in this study (Table 4). Unidentified direct interactions between these two organisms might occur in the subgingival environment. However, co-infection of *T. forsythia* and *P. gingivalis* showed a synergistic effect in a murine abscess formation model (Yoneda et al. 2001). It has also been suggested that the proteolytic activity of *P. gingivalis* is important in increasing the virulence of *T. forsythia* (Holt & Ebersole 2005). The results of the multivariate analysis indicated that the WB21 group presented a reduction of *P. gingivalis* compared with the placebo group (4W: OR = 8.00; 95% CI = 0.72–88.6; $p = 0.09$, and 8W: OR = 6.66; 95% CI = 0.39–113; $p = 0.19$, respectively), although a significant difference was not found between the groups (Table 4). A slight suppression of *P. gingivalis* might add a synergistic effect to the interaction of *L. salivarius* WB21 with *T. forsythia*, resulting in a significant reduction in the test group.

The number of periodontopathic bacteria in both supragingival and subgingival plaque were also decreased in the placebo group in this study. *L. salivarius* WB21 intake only showed a significant reduction in the number of subgingval periodontopathic bacteria at 4W, with gradually decreased bacterial counts in both groups (Fig. 3). Similar improvements of the clinical parameters including PlI were also observed for the placebo group subjects, although neither group had oral hygiene instructions before or during the experimental intervention (Shimauchi et al. 2008). Thus, the reduced plaque accumulation may have induced ''placebo effects'' on subgingival microbiota in these subjects that partially masked the probiotic effects by *L. salivarius* WB21. This phenomenon was considered to be caused by an attention bias (a Hawthorne effect), which is a systematic change in behaviour in study participants under observation. Niv et al. (2005) reported the improvement of irritable bowel syndrome symptoms of placebo group patients in their RCT using *Lactobacillus reuteri* as probiotics, suggesting the presence of a strong placebo effect. However, the present study suggests that probiotic effects of *L. salivarius* WB21 do exist overlaying the perceived placebo effect in the test group.

It was also of concern whether the daily intake of *L. salivarius* WB21 may increase the risk of dental caries caused by lactobacilli-producing lactic acid, although no adverse effects were observed during the intervention until the 8W follow-up. Matsumoto et al. (2005) reported that superinfection of probiotic *L. salivarius* LR1952R with *S. mutans* MT8148 significantly increased caries scores compared with *S. mutans* MT8148 alone in rat experimental models, suggesting that close attention is necessary over the intake of acid-tolerant *L. salivarius* WB21 in the long term to monitor the caries risk.

In the present study, it was shown that *L. salivarius* WB21 administration suc-

cessfully decreased the numerical sum of five periodontopathic bacteria in subgingival plaque at 4W. A life-long need for plaque control arises for the treatment and prevention of periodontal diseases. As the intake of *L. salivarius* WB21 tablets is an easily introducible approach, probiotics may be provided as homecare supplements for preventing periodontal diseases. It is also well known that the re-emergence of periodontal pathogens is correlated with a lack of clinical improvement and an increased risk for disease relapse (Haffajee et al. 1997). Therefore, administration of *L. salivarius* WB21 probiotics as an adjunct to mechanical debridement might be an effective approach for the treatment of periodontitis. Probiotic therapy may be a biological approach for controlling oral microbiota to induce a beneficial shift away from pathogens (Persson 2005). It was also concluded that further studies are necessary in order to provide biological plaque control as an armamentarium of treatment options for periodontal diseases.

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Clinical Relevance

Scientific rationale for the study: Lactobacilli are frequently used as probiotics to induce a beneficial effect on human health. However, little is known about the effect of probiotics on periodontal diseases.

Principal findings: After probiotic intervention with *L. salivarius* WB21 for 8W in healthy volunteers, the numerical sum of five selected periodontopathic bacteria in the test group was decreased significantly compared with the control group in subgingival plaque at 4W.

Practical implication: The present study suggests that a probiotic intervention could be a useful tool for the improvement of periodontal health.