

Probiotic Compared with Standard Milk for High-caries Children: A Cluster Randomized Trial

G. Rodríguez¹, B. Ruiz¹, S. Faleiros¹, A. Vistoso¹, M.L. Marró¹,
J. Sánchez¹, I. Urzúa¹, and R. Cabello¹

Abstract

The aim of this study was to compare milk supplemented with probiotic lactobacilli with standard milk for the increment of caries in preschool children after 10 mo of intervention. The study was a triple-blind, placebo-controlled randomized trial. Participants were children aged 2 and 3 y ($n = 261$) attending 16 nursery schools in a metropolitan region in Chile. Nursery schools were randomly assigned to 2 parallel groups: children in the intervention group were given 150 mL of milk supplemented with *Lactobacillus rhamnosus* SPI (10^7 CFU/mL), while children in the control group were given standard milk. Interventions took place on weekdays for 10 mo. Data were collected through a clinical examination of participants. The primary outcome measure was the increment of caries in preschool children. This was assessed using the International Caries Detection and Assessment System (ICDAS). The dropout rate was 21%. No differences in caries prevalence were detected between the groups at baseline ($P = 0.68$). After 10 mo of probiotic intake, the caries prevalence was 54.4% in the probiotic group and 65.8% in the control group. The percentage of new individuals who developed cavitated lesions (ICDAS 5-6) in the control group (24.3%) was significantly higher than that in the probiotic group (9.7%). The increment of dental caries showed an odds ratio of 0.35 ($P < 0.05$) in favor of the probiotic group. At the cavitated lesion level, the increment of new caries lesions within the groups showed 1.13 new lesions per child in the probiotic group compared with 1.75 lesions in the control group ($P < 0.05$). The probiotic group showed an increment of 0.58 ± 1.17 new lesions compared with 1.08 ± 1.70 new lesions observed in the control group. The difference in caries increment was significant at the cavitated lesion level ($P < 0.01$). In conclusion, the regular long-term intake of probiotic-supplemented milk may reduce caries development in high-caries preschool children (ClinicalTrials.gov: NCT01648075).

Keywords: preschool children, *Lactobacillus rhamnosus* SPI, bacteriotherapy, caries prevention, early childhood caries, supplemented milk

Introduction

Early childhood caries is a prevalent public health problem in developing countries, and it still poses a significant burden on health services worldwide. Regional data (MINSAL 2010) have shown the following: a prevalence of 17% in 2-y-old children, an increased prevalence of 48% at 4 y of age, and 70% prevalence in 6-y-old children according to World Health Organization (WHO) criteria.

When dental caries occurs at early stages in life, it may result in the complete destruction of teeth and cause variable degrees of pain, acute infections, nutritional deficiencies, and speech and learning difficulties and may require expensive treatment (American Academy of Pediatric Dentistry 2011). The cumulative damage is demonstrated as a high number of affected teeth and untreated caries lesions (Tinanoff et al. 2002). Children with caries lesions are at risk of developing new caries lesions in the future because of their predictive risk value in permanent dentition (Peretz et al. 2003). Dental caries occurs as a result of an imbalance in the resident oral microflora due to an increase in highly cariogenic bacteria associated

with frequent intraoral conditions of low pH (Marsh 2010; Takahashi and Nyvad 2011).

Probiotics are live microorganisms that confer a health benefit to the host (WHO/FAO 2002). Several mechanisms of action of probiotics have been proposed, including a variety of combined local and systemic effects that involve adhesion, co-aggregation, competitive inhibition, production of organic acids and bacteriocin-like compounds, and immune modulation (Teughels et al. 2008; Twetman and Keller 2012). These mechanisms can also stimulate nonspecific cellular and humoral responses (Bonifait et al. 2009). Clinical studies have shown that strains such as *Lactobacillus rhamnosus* GG, *L. acidophilus* La-5, *L. brevis* CD2, *Bifidobacterium animalis*

¹Department of Restorative Dentistry, University of Chile, Santiago, Chile

Corresponding Author:

R. Cabello, Department of Restorative Dentistry, University of Chile, Sergio Livingstone Pohlhammer 943, Independencia, Santiago, Chile.
Email: rcabello@odontologia.uchile.cl

subsp. *lactis* BB-12, *B. longum*, and *Saccharomyces cerevisiae* produce a reduction in mutans streptococci (MS) counts in plaque and/or saliva (Cagetti et al. 2013).

Six clinical trials have investigated the effect of probiotic administration on caries prevalence as an endpoint. Five of them were conducted in children (Näse et al. 2001; Stecksén-Blicks et al. 2009; Hasslöf et al. 2013; Taipale et al. 2013; Stenstrom et al. 2014) and one in adults (Pettersson et al. 2011). Four studies reported a reduction in caries occurrence after probiotic exposure compared with control groups (Näse et al. 2001; Stecksén-Blicks et al. 2009; Pettersson et al. 2011; Stenstrom et al. 2014).

Näse et al. (2001) found a reduction, albeit not significant, of caries increment in children after a 7-mo intake period of milk supplemented with *L. rhamnosus* GG. Another study reported that the ingestion of milk supplemented with *L. rhamnosus* LB21 and 2.5 mg/L of fluoride for 21 mo reduced caries occurrence. However, the authors stated that it was difficult to establish whether the effects were solely due to the probiotic strain used or whether the presence of fluoride in their study might have affected the results (Stecksén-Blicks et al. 2009). Taipale et al. (2013) did not find differences in the occurrence of caries between groups of 4-y-old children according to the International Caries Detection and Assessment System (ICDAS). Hasslöf et al. (2013) reported that the early administration of a cereal supplemented with *L. paracasei* F19 did not affect the occurrence of dental caries and MS or lactobacilli counts. Using a different strain, Stenstrom et al. (2014) reported that oral supplementation with *L. reuteri* in women during their last month of pregnancy, as well as children through their first year of life, was associated with reduced caries prevalence and gingivitis.

Although differences in methodology were found between the available clinical studies and making direct comparisons between their results is difficult, these findings suggest that specific probiotic lactobacilli can promote dental health. There are no available data about caries occurrence when probiotic bacteria are administered on a daily basis in high-caries populations during early childhood. The aim of this study was to compare milk supplemented with probiotic lactobacilli with standard milk for caries increment in preschool children after 10 mo of intervention.

Materials and Methods

Participants

Two- to 3-y-old children participated in this trial with an allocation ratio of 1:1. Participants were recruited from 16 nursery schools from the Integra Foundation located in the northwestern area of a metropolitan region. The Integra Foundation focuses on the education of the most socially vulnerable children in Chile. According to epidemiological data, these children represent the group with the highest risk of dental caries in the Chilean population (MINSAL 2010). Informed consent was obtained in writing from the children's

parents or legal guardian. Children whose families refused to participate in this study were given their school milk in its usual format.

The main sources of fluoride exposure to participants were water fluoridation and tooth brushing. The northwestern area of a metropolitan region in Chile is engaged in a tap water artificial fluoridation program (0.62 ppm). All the children underwent supervised tooth brushing with fluoride toothpaste (450–500 ppm) at least once a day at nursery school. Oral health care was provided within the public health service through its primary care network. Restorative dental treatment was received by less than 10% of the children. This figure is in keeping with the national rate of children who receive restorative dental treatment.

In order to be included in the study, the following inclusion criteria were applied: healthy children (based on parents reporting on their children's medical history) with or without cavitated caries lesions and children without milk intolerance or food allergies.

Study Design, Sample Size, Randomization, and Blinding

The study was designed as a cluster randomized, triple-blind, placebo-controlled, 2-arm trial, and interventions were performed between August 2012 and July 2013. The published protocol makes reference to an 18-mo period. Taking into account the Chilean school year format, we had to modify the original 18-mo period to a 10-mo period in order to accommodate for this (NCT01648075, <http://www.clinicaltrials.gov>). Using random numbers, one of the authors (G.R.) assigned the different nursery schools either to the intervention group or to the control group. The nursery schools were allocated a green or yellow code in order to conceal their identity. The code was kept by the lead author (G.R.) and was not unveiled until all data were analyzed. None of the researchers and clinicians, nor the personnel or families of participants at the nursery schools, knew whether the children received the control or the probiotic-supplemented milk.

The intracluster correlation coefficient was determined to be 0.01 and accounted for the relatedness of the clustered data. Determination of the sample size was estimated on the basis of epidemiological data on the caries prevalence of the 2-y-old children living in the area (mean [\pm standard deviation] dmft, 0.54 ± 2.80) (MINSAL 2010). Cluster numbers were set for 16 nursery schools. It was estimated that 120 children needed to be allocated to each arm in order to detect a mean clinical reduction of 1.2 ± 2.8 ($d_{\text{icdas5-6}}$ -mft) caries lesions ($\alpha = 0.05$ and $\beta = 0.20$). The estimated proportion of dropouts was determined to be 10% per year. Therefore, 10% of oversampling was performed in order to compensate for this. There are 101 Integra nursery schools and nearly 40 private ones in this area. Of the 101 Integra nursery schools, 16 were randomly selected in 2 separate phases: districts were first selected at random, followed by random selection of the nursery schools.

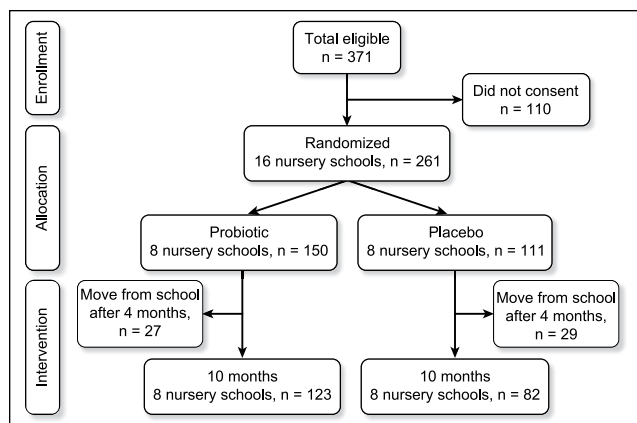


Figure. Flow chart of the randomization and dropout of participants throughout the 10-mo intervention period.

This study was conducted in accordance with the guidelines laid out in the Declaration of Helsinki. All procedures involving patients were approved by the Ethics Committee of the Faculty of Dentistry at the University of Chile (certificate # 2011/14; NCT01648075, <http://www.clinicaltrials.gov>).

Intervention

Children were given 150 mL of medium-fat milk during their afternoon break. According to the published protocol, the use of skimmed milk was intended. However, in the study, medium-fat milk was used because this is the official type of milk used by schools under the National Nutrition Program. Milk was prepared by the nursery school staff by adding a 500-g bag of powdered milk (Macro Food, Santiago, Chile) to 5 L of water at 40 °C that had been previously boiled. After stirring the preparation, a sachet of probiotics was added to the milk to attain a final 10^7 -CFU/mL concentration of *L. rhamnosus* SP1 (Sacco, Cadorago, Italy) for the probiotic group. The placebo sachet for the control group only contained medium-fat milk. The probiotic-supplemented milk and the placebo milk were prepared and given to the children only on weekdays. During the intervention period, samples of milk were taken, and microbiological tests were performed to assess the presence of probiotic bacteria. Children were exposed to this intervention for a total of 40 wk.

Check of Compliance

Staff at the nursery schools filled in a logbook every day with information regarding the attendance of each child or his or her absence due to sickness or any other circumstances.

Clinical Examination and Data Collection

Clinical dental examinations were performed at baseline and at the end of the study. Two dentists, using an artificial light, a mouth mirror, and a WHO periodontal probe, examined all of the children at the nursery schools. ICDAS criteria were used for the visual and tactile detection of dental caries lesions and

for describing the severity of lesions (Ismail et al. 2007). Training of examiners started with an intensive review of the ICDAS criteria. Initially, examiners were trained by undergoing a thorough review of the literature and were calibrated by carrying out repeated evaluations of clinical pictures. In addition, an examination of 30 patients as part of their calibration was conducted in which an expert clinician formerly calibrated by the ICDAS Committee was used as the gold standard for the reliability of assessments. Every single ICDAS score was used. Intraexaminer and interexaminer reliability values resulted in κ values of 0.72 (0.715 and 0.726 were obtained by the 2 examiners) and 0.71 (0.70 and 0.719), respectively. ICDAS criteria were used in this study to describe the severity of dental caries lesions. ICDAS codes 2 to 6 were used to measure caries increment. Codes 5 and 6 were considered an independent group in order to show the caries increment for obvious dental caries and to simulate what the increment would have been in terms of WHO criteria.

Outcome Measures

The primary outcome measure of the present study was the increment of caries in preschool children.

Statistical Analysis

Differences between groups were analyzed for statistical significance using the Pearson χ^2 test for categorized/dichotomized variables and an analysis of variance for the interval-level variables. $P < 0.05$ was considered statistically significant. Data were analyzed using STATA (version 11.0; StataCorp, College Station, TX, USA).

Results

The number of children who participated is illustrated in the flow chart (Fig.). The baseline characteristics of the children in the intervention group and the control group are presented in Table 1. There were no statistically significant differences between the groups. Also, there were no significant differences between the children who remained in the study and those who dropped out of the study.

Effect on Dental Caries

Caries data at baseline and at the end of the study are shown in Table 2. The caries prevalence of the children in the intervention group and the control group was balanced at baseline in terms of both the level of cavitated ($d_{\text{icdas}5-6\text{mft}}$) ($P = 0.43$) and noncavitated ($d_{\text{icdas}2-6\text{mft}}$) carious lesions ($P = 0.68$).

At the end of this study, results from the examinations showed caries prevalence (ICDAS 2–6) in 54.4% of the probiotic group and in 65.8% of the control group. The number of new participants who developed cavitated lesions (ICDAS 5–6) after the intervention period was significantly higher in the control group (24.3%) than in the probiotic group (9.7%). The increment of prevalence shows an odds ratio (OR) of 0.35 ($P < 0.05$) in favor of the probiotic group.

Table 1. Baseline Characteristics of the Children Who Entered the Study.

Characteristic	Probiotic Group	Control Group	Dropouts
Female sex, %	50	53	49
Age, mean \pm SD, y	2.89 \pm 0.31	2.97 \pm 0.29	2.92 \pm 0.28
ICDAS 2–6 >0, %	39.3	42.3	39.3
ICDAS 5–6 >0, %	23.3	22.5	25.0
$d_{\text{icdas}2-6}$ mft, mean \pm SD	1.62 \pm 2.51	1.69 \pm 2.75	1.28 \pm 2.15
$d_{\text{icdas}5-6}$ mft, mean \pm SD	0.67 \pm 1.71	0.69 \pm 1.56	0.75 \pm 1.67

ICDAS, International Caries Detection and Assessment System; SD, standard deviation.

Table 2. Dental Caries in the Intervention Group ($n = 123$) and Control Group ($n = 82$) at Baseline and at the End of the Study.

	Probiotic Group	Control Group	Odds Ratio	ICC
Nursery schools, n	8	8		
Participants, n	123	82		
ICDAS 2–6 >0, baseline, %	39.3	42.3	0.94 (0.62–1.44)	
ICDAS 2–6 >0, 12 mo, %	54.4	65.8	0.76 (0.51–1.14)	
Δ ICDAS 2–6 >0, 12 mo – baseline, %	16.2	20.8	0.76 (0.38–1.51)	
ICDAS 5–6 >0, baseline, %	23.3	22.5	1.17 (0.68–2.01)	
ICDAS 5–6 >0, 12 mo, %	33.3	45.1	0.71 (0.43–1.15)	
Δ ICDAS 5–6 >0, 12 mo – baseline, %	9.7	24.3	0.35 (0.16–0.79)^a	
$d_{\text{icdas}2-6}$ mft, baseline, mean \pm SD	1.62 \pm 2.51	1.69 \pm 2.75		0.03
$d_{\text{icdas}2-6}$ mft, 12 mo, mean \pm SD	2.86 \pm 3.64	3.58 \pm 3.66		0.01
$\Delta d_{\text{icdas}2-6}$ mft, 12 mo – baseline, mean \pm SD	1.13 \pm 1.94	1.75 \pm 2.37		0.03^b
$d_{\text{icdas}5-6}$ mft, baseline, mean \pm SD	0.67 \pm 1.71	0.69 \pm 1.56		0.03
$d_{\text{icdas}5-6}$ mft, 12 mo, mean \pm SD	1.30 \pm 2.31	1.68 \pm 2.69		0.04
$\Delta d_{\text{icdas}5-6}$ mft, 12 mo – baseline, mean \pm SD	0.58 \pm 1.17	1.08 \pm 1.70		0.02^a

ICC, intraclass correlation coefficient; ICDAS, International Caries Detection and Assessment System; SD, standard deviation.

Boldface indicates statistical significance.

^a $P < 0.01$.

^b $P < 0.05$.

The mean caries prevalence ($d_{\text{icdas}2-6}$ mft) was 2.86 ± 3.64 and 3.58 ± 3.66 after 12 mo for the probiotic group and control group, respectively. The caries increment is expressed as Δ mft. The increment of new caries lesions within the groups ($\Delta d_{\text{icdas}2-6}$ mft, 12 mo – baseline) showed 1.13 new lesions per child in the probiotic group versus 1.75 lesions in the control group; the difference in increment was statistically significant ($P < 0.05$). When examined at the cavitated lesion level (ICDAS 5–6) after the 10-mo intervention period, the probiotic group exhibited an increment of 0.58 ± 1.17 new lesions per child compared with 1.08 ± 1.70 new lesions in the control group. The difference in caries increment was also significant at the cavitated lesion level ($P < 0.01$).

After multivariate analysis, it was confirmed that probiotic intake showed a statistically significant OR of 0.32 ($P = 0.006$), indicating that individuals from the probiotic group have a lower probability of manifesting caries increment (cavitated lesions) during the follow-up period (Table 3). The number needed to treat was 8 (95% confidence interval, 4.1–26.5). No adverse effects were reported by parents during the intervention period.

Discussion

This clinical trial was performed to test whether the daily consumption of milk supplemented with the probiotic bacteria *L. rhamnosus* SP1 reduces the caries increment compared with a

Table 3. Multivariate Analysis of Δ ICDAS 5–6 >0 (12 mo – Baseline).

Variable	Odds Ratio	P Value	95% Confidence Interval
Probiotic group	0.32	0.006	0.14–0.72
Sex	0.84	0.657	0.40–1.78
Age at 12 mo	1.54	0.450	0.49–4.79

ICDAS, International Caries Detection and Assessment System.

Boldface indicates statistical significance.

daily intake of standard milk as a placebo in high-caries pre-school children. The dropout rate was 21%. The main reason for participants leaving the study was related to children moving from one nursery school to another due to family-associated needs. The expected attrition rate was 10% per year. However, the observed attrition rate was 21% after 10 mo (26% for the control group and 18% for the experimental group). This high attrition rate could have resulted in a source of bias as individuals who left the study may have different characteristics than those who remained in the study. To assess this issue, a baseline data analysis was conducted. No significant differences in the baseline caries data were found between the children who dropped out and those who attended the final examination. Dropouts were almost equally distributed in the 2 study groups. On the other hand, final oversampling after enrollment was 12% in the control group and 51% in the experimental group.

Oversampling in the experimental group was higher because schools differed in the number of pupils that they had.

Cluster randomization for this trial provided protection against contamination across groups within the study setting. This randomized approach was performed at the patient level and accounted for the use of intracluster correlation, which increases the statistical power of the analysis.

According to the logbooks, which teachers kept in each classroom, compliance with the study protocol was acceptable. The intervention lasted for 190 d. Children from the probiotic group attended 76% of those days, and children from the control group attended 81%. It should be noted that no intervention or placebo milk was given during the holiday season (2 wk in July and all of January and February).

The rationale behind the selection of the probiotic strain used in this study was based on previous findings showing that *L. rhamnosus* exhibits antagonistic activity against cariogenic bacteria (Simark-Mattsson et al. 2007) and also because it had been used in previous clinical trials, resulting in a reduction of caries risk (Näse et al. 2001) as well as exhibiting a decrease in dental caries in preschool children (Stecksén-Blicks et al. 2009). *L. rhamnosus* SP1 has also shown good performance as a probiotic strain in the manufacturing processes of several functional beverages (Coda et al. 2011), Fior di Latte cheese (Minervini et al. 2012), and yogurt (Pavón et al. 2014).

Two dentists, who were blind to the process of group allocation, underwent intraexaminer and interexaminer calibration before baseline examinations. The interexaminer and intraexaminer agreement values were acceptable (Landis and Koch 1977; Dawson and Trapp 2004). The reliability of the staff's logbook of milk consumption and attendance was considered high because it was updated on a daily basis and had the support of an external observer who controlled correct recording of the logbooks once a week. Nevertheless, proof of good compliance to ensure daily ingestion of the probiotic strain was not confirmed through fecal sampling.

Our finding, that probiotic-supplemented milk reduced caries prevalence in this group with a high caries rate, is in line with those of Näse et al. (2001), Stecksén-Blicks et al. (2009), Petersson et al. (2011), and Stensson et al. (2014), as in all of them, a significant reduction of caries lesions or caries risk factors was found. Nevertheless, the study by Petersson et al. (2011) was conducted in adults, and Stensson et al. (2014) found differences only at the age of 9 y. Stecksén-Blicks et al. (2009) added fluoride to milk, making it impossible to evaluate the probiotic effect alone. Our results differ from those of Taipale et al. (2013) and Hasslöf et al. (2013), as early supplementation of different probiotic strains did not produce a significant caries reduction measured at age 4 and 9 y, respectively.

A novel component of this study is that the probiotic strain was lyophilized and packed in a sachet, ready for adding it to powdered milk. With this method, there is no need to comply with cold chain requirements that would be essential to maintain other probiotic-supplemented dairy vehicles.

A proposed mechanism of action that could explain the beneficial outcome observed in this research project is that the probiotic strain interferes with and modifies the oral biofilm,

shifting the oral ecology toward more beneficial bacteria that produce fewer organic acids and thus maintaining plaque and saliva pH in a state of equilibrium. However, it is important to note that as seen in previous laboratory and short-term interventional studies, probiotic lactobacilli were found to be only transiently present in saliva during and shortly after interventions (Petti et al. 2001; Yli-Knuutila et al. 2006; Caglar et al. 2009). Products containing *L. rhamnosus* GG show rapid clearance from the mouth when probiotic intake stops. Therefore, clinical trials such as this one must rely on the daily intake of probiotics in order to achieve a reduction in caries.

Interestingly enough, despite the fact that the effects of probiotics on the occurrence of gastrointestinal disorders were beyond the scope of this study, probiotic intervention had no reported effects according to the records of the physician who monitored both groups throughout the intervention. Similarly, no adverse effects were reported by the parents. Participants were fully masked to the type of milk they consumed, and the examiners and statistician used color codes.

The limitations of this trial are mainly related to 3 aspects. First of all, randomization of the nurseries might induce a risk of bias, although the clusters were very similar. Second, the attrition rate of the study should be considered when looking at the results. Finally, a limitation was the short-term nature of the follow-up period.

In conclusion, the findings of the present study show that the regular intake of probiotic-supplemented milk reduces the caries increment in preschool children. Further studies are needed to clarify the mechanisms of action of probiotics within the oral cavity and to evaluate the cost-effectiveness of milk supplementation as a matter of public health in order to address the burden of dental caries in high-caries populations.

Author Contributions

G. Rodríguez, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; B. Ruiz, contributed to data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; S. Faleiros, contributed to data acquisition and analysis, critically revised the manuscript; A. Vistoso, M.L. Marró, J. Sánchez, I. Urzúa, contributed to data acquisition, critically revised the manuscript; R. Cabello, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This study was funded by CONICYT FONIS SA11I2035 (Government of Chile). The authors thank Dr. Diego Jazanovich for his revisions of the article. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

- American Academy of Pediatric Dentistry. Policy on early childhood caries (ECC): classifications, consequences and preventive strategies. 2011. Chicago (IL): American Academy of Pediatric Dentistry; [accessed

- 2015 April]. http://www.aapd.org/media/Policies_Guidelines/P_ECC_Classifications.pdf.
- Bonifait L, Chandad F, Grenier D. 2009. Probiotics for oral health: myth or reality? *J Can Dent Assoc.* 75(8):585–590.
- Cagetti MG, Mastroberardino S, Milia E, Cocco F, Lingström P, Campus G. 2013. The use of probiotic strains in caries prevention: a systematic review. *Nutrients.* 5(7):2530–2550.
- Çaglar E, Topcuoglu N, Cildir SK, Sandalli N, Kulekci G. 2009. Oral colonization by *Lactobacillus reuteri* ATCC 55730 after exposure to probiotics. *Int J Paediatr Dent.* 19(5):377–381.
- Coda R, Rizzello CG, Trani A, Gobbetti M. 2011. Manufacture and characterization of functional emmer beverages fermented by selected lactic acid bacteria. *Food Microbiol.* 28(3):526–536.
- Dawson B, Trapp RG. 2004. Basic & clinical biostatistics. LANGE basic science. New York (NY): McGraw-Hill. Chapter 5; p. 115–117.
- Hasslöf P, West CE, Videhult FK, Brandelius C, Stecksén-Blicks C. 2013. Early intervention with probiotic *Lactobacillus paracasei* F19 has no long-term effect on caries experience. *Caries Res.* 47(6):559–565.
- Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, Pitts NB. 2007. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dent Oral Epidemiol.* 35(3):170–178.
- Landis JR, Koch GG. 1977. The measurement of observer agreement for categorical data. *Biometrics.* 33(1):159–174.
- Marsh PD. 2010. Microbiology of dental plaque biofilms and their role in oral health and caries. *Dent Clin North Am.* 54(3):441–454.
- Minervini F, Siragusa S, Faccia M, Dal Bello F, Gobbetti M, De Angelis M. 2012. Manufacture of Fior di Latte cheese by incorporation of probiotic lactobacilli. *J Dairy Sci.* 95(2):508–520.
- MINSAL. Diagnóstico de salud bucal. 2010. Chile: Ministry of Health; [accessed 2015 April]. <http://web.minsal.cl/portal/url/item/7dc33df0bb34ec58e04001011e011c36.pdf>.
- Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, Korpela R, Meurman JH. 2001. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res.* 35(6):412–420.
- Pavón YL, Lazzaroni SM, Sabbag NG, Rozycki SD. 2014. Simultaneous effects of gelatin and espina corona gum on rheological, physical and sensory properties of cholesterol-reduced probiotic yoghurts. *Int J Food Sci Technol.* 49(10):2245–2251.
- Peretz B, Ram D, Azo E, Efrat Y. 2003. Pre-school caries as an indicator of future caries: a longitudinal study. *Pediatr Dent.* 25(2):114–118.
- Petersson LG, Magnusson K, Hakestam U, Baigi A, Twetman S. 2011. Reversal of primary root caries lesions after daily intake of milk supplemented with fluoride and probiotic lactobacilli in older adults. *Acta Odontol Scand.* 69(6):321–327.
- Petti S, Tarsitani G, D'Arca AS. 2001. A randomized clinical trial of the effect of yoghurt on the human salivary microflora. *Arch Oral Biol.* 46(8):705–712.
- Simark-Mattsson C, Emilson CG, Håkansson EG, Jacobsson C, Roos K, Holm S. 2007. *Lactobacillus*-mediated interference of mutans streptococci in caries-free vs. caries-active subjects. *Eur J Oral Sci.* 115(4):308–314.
- Stecksén-Blicks C, Sjöström I, Twetman S. 2009. Effect of long-term consumption of milk supplemented with probiotic lactobacilli and fluoride on dental caries and general health in pre-school children: a cluster-randomized study. *Caries Res.* 43(5):374–381.
- Stensson M, Koch G, Coric S, Abrahamsson TR, Jenmalm MC, Birkhed D, Wendt LK. 2014. Oral administration of *Lactobacillus reuteri* during the first year of life reduces caries prevalence in the primary dentition at 9 years of age. *Caries Res.* 48(2):111–117.
- Taipale T, Pienihäkkinen K, Alanen P, Jokela J, Söderling E. 2013. Administration of *Bifidobacterium animalis* subsp. *lactis* BB-12 in early childhood: a post-trial effect on caries occurrence at four years of age. *Caries Res.* 47(5):364–372.
- Takahashi N, Nyvad B. 2011. The role of bacteria in the caries process: ecological perspectives. *J Dent Res.* 90(3):294–303.
- Teughels W, Van Essche M, Sliepen I, Quirynen M. 2008. Probiotics and oral healthcare. *Periodontol* 2000. 48:111–147.
- Tinanoff N, Kanellis MJ, Vargas CM. 2002. Current understanding of the epidemiology mechanisms, and prevention of dental caries in pre-school children. *Pediatr Dent.* 24(6):543–551.
- Twetman S, Keller MK. 2012. Probiotics for caries prevention and control. *Adv Dent Res.* 24(2):98–102.
- WHO/FAO. Guidelines for the evaluation of probiotics in food. 2002. London, Ontario (Canada): WHO and FAO; [accessed 2015 April]. www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.
- Yli-Knuutila H, Snäll J, Kari K, Meurman JH. 2006. Colonization of *Lactobacillus rhamnosus* GG in the oral cavity. *Oral Microbiol Immunol.* 21(2):129–131.