

Recent Achievements in Preventive Dentistry by Introducing a New Probiotic Toothpaste

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ABSTRACT

Artificially synthesized probiotic from *Lactobacillus* strain, contained in the tested toothpaste, led to an innovative approach in preventive dentistry. A new concept resulting from this research can be explained due to possible mechanisms of action of probiotic bacteria, according to which equilibrium of hostile bacterial flora is achieved by mechanical elimination of cariogenic bacteria from the mouth. This research was conducted during a 4-week period on a randomly selected Croatian sample of 50 participants, with the aim to investigate the efficacy of the first probiotic toothpaste ever produced in Croatia. CRT tests (Ivoclar Vivadent AG, FL-9494 Schaan / Liechtenstein) were used and individually administered to each participant to assess the number of *Streptococci* and *Lactobacilli*. Saliva samples were tested before, as well as 2 and 4 weeks after using the tested toothpaste. After having had obtained detailed information on the research protocol, participants signed informed consent, and strictly following the instructions, brushed their teeth exclusively using the tested toothpaste and toothbrush of the same manufacturer over the 4 week period. Statistical results obtained after 4 weeks of using the probiotic toothpaste showed significant reduction in the number of participants who, prior to commencing the study, were diagnosed a high number of cariogenic bacteria. The number of participants with the high number of streptococci was significantly reduced from 78.4 to 26.5%, as well as the number of participants with the high number of *Lactobacilli*, which significantly dropped down from 52.9 to 26.5%. The results indicate a significant efficacy of the tested toothpaste, which can be attributed to the effect of the contained synthesized probiotic substance. Therefore, this research reveals a new achievement in innovative technologies, based on which probiotics can be used with purpose of maintaining balance of bacterial flora within the oral cavity, particularly in high risk groups who are more prone to developing caries.

Key words: toothpaste, probiotics, CRT tests

Introduction

The recent achievements in oral microbiology have proven a diverse range of more than 700 bacterial species which are incorporated in the dental biofilm (plaque) in the form of colonised pellicle coat^{1,3}. In the presence of fermentable carbohydrates, these bacteria produce acids that commence a localised demineralization of the tooth hard tissue, finally resulting in dental caries which is known as the most common and major oral disease caused by the oral microbial flora^{4,5}. With respect to the latest concept of dental diseases caused due to oral microbial interactions, an important shift has suggested a new approach for controlling dental caries via modulating the oral microbial ecology. This approach suggests the selective inhibition of oral pathogens or modulation

of the microbial composition of dental plaque in order to control microbial pathogenesis. It could be achieved by applying mechanical means and improved by introducing toothpastes and mouthrinses which involve probiotics as tremendously effective in preventing oral diseases and maintaining oral health. Following this updated concept, innovative technology in dentistry has presented a newly synthesized product made from a probiotic microorganism strain *Lactobacillus paracasei*, known as pro-t-action, which represents a therapeutic innovation that can serve in the battle against caries. The active ingredient in pro-t-action was proved to bind to and eliminate causing bacteria caries from the mouth. associated salivary microbial counts, i.e. *Streptococcus mutans* and *Lactoba-*

cillus, which are normally present in the mouth. The objective was also to evaluate a potential influence of the same toothpaste on the saliva buffer capacity. Following this updated concept, innovative technology in dentistry has presented a newly synthesized product made from a probiotic microorganism strain *Lactobacillus paracasei*, known as pro-t-action, which represents a therapeutic innovation that can serve in the battle against caries. The active ingredient in pro-t-action was proved to bind to and eliminate causing bacteria caries from the mouth. associated salivary microbial counts, i.e. *Streptococcus mutans* and *Lactobacillus*, which are normally present in the mouth. The objective was also to evaluate a potential influence of the same toothpaste on the saliva buffer capacity associated salivary microbial counts, i.e. *Streptococcus mutans* and *Lactobacillus*, which are normally present in Following this updated concept, innovative technology in dentistry has presented a newly synthesized product made from a probiotic microorganism strain *Lactobacillus paracasei*, known as pro-t-action, which represents a therapeutic innovation that can serve in the battle against caries. The active ingredient in pro-t-action was proved to bind to and eliminate causing bacteria caries from the mouth. associated salivary microbial counts, i.e. *Streptococcus mutans* and *Lactobacillus*, which are normally present in the mouth. The objective was also to evaluate a potential influence of the same toothpaste on the saliva buffer capacity.

Materials and Methods

A randomly selected sample of 50 volunteers who were 4- and 5-year attendants of the School of Dental Medicine of the University of Zagreb, aged between 20 and 24 years, were included in this 4-week clinical trial. All subjects were equally divided by genders. The number of bacteria present in the mouth was evaluated by using a specific method, i.e. the standard CRT evaluation, which has proved to be an objective method for detection of bacterial counts. At the screening, all participants represented within the normal range and showed no signs of abnormality or extremely high or low bacterial counts. Therefore, a placebo group was not introduced in this study. Prior to commencing the study, all participants were explained the purpose of the study as well as all the requirements established by the protocol criteria and the product itself. After giving consent, they agreed on volunteering in the study. The toothpaste had previously been clinically tested in The Pliva Research Institute in Zagreb and the Dental Clinic at the Johannes Gutenberg University in Mainz and approved of by the Croatian Department of Health. It had been produced according to ISO 9001/14001 integrated quality normatives, which the company (Neva, Croatia) first introduced in Croatia. The raw materials were obtained from western European suppliers and were all EU quality certificated and met health regulations. The products had proved free of harmful abrasive materials and hadn't been tested on animals. All participants were supplied by probiotic tooth-

pastes (Pro-T-Action, Plidenta, Neva, Croatia), which contained an active substance i.e. pro-t-action, and by medium hard toothbrushes as well (Plidenta, Neva, Croatia). They were instructed to brush their teeth thoroughly 2 times a day and were asked to use dental floss. In the period of duration of the study, subjects were restrained from using any other toothpaste or oral product from another supplier. They were strongly suggested to remain within the usual dietary protocol. Any participant who might have represented with an allergic reaction that potentially could be related to the labeled toothpaste, was supposed to be excluded from the study immediately. In medical history, exclusion criteria referred to any major chronic or systemic diseases that might have interfered with the content of the labeled product. Subjects with the history of smoking, immune system incompetency, and gastrointestinal disturbances were excluded as well. Dental history exclusion criteria involved gross caries lesions, extensive prosthetic work, tooth abnormalities (such as dentinogenesis or amelogenesis), odontodysplasia and periodontal diseases. Only one participant was excluded in parts of duration of the study due to combining the labeled toothpaste with another oral hygiene product. By applying a standard CRT (Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein) test evaluation, screening of the participants was accomplished by detecting the number of cariogenic bacteria (*Streptococci* and *Lactobacilli*) at the initial visit. Based on the bacterial count at the initial visit, all participants were grouped either in a: A/ group with a low *Streptococcus mutans* and *Lactobacillus* count ($<10^5$), or B/ group with a high *Streptococcus mutans* and *Lactobacillus* count ($\geq 10^5$). Prior to applying CRT tests, individual samples of stimulated saliva were used to obtain buffer capacity for each subject. The rest of the stimulated saliva samples were further individually tested by using CRT tests. Upon collecting stimulated saliva from an individual subject, each CRT sample was kept in the thermostat at 37 °C over the 48 hour period, after which time the number of *Streptococci* and *Lactobacilli* were counted. CRT tests were individually applied to each subject at the 1st, 2nd and 4th week check-ups, during which time the subjects were instructed to brush their teeth following the required protocol. Buffer capacity (i.e. pH value) was measured at the 2nd and 4th week check-up intervals.

Statistics

Statistical analysis was performed by using IBM SPSS Statistics 20 for Windows. For the purpose of the study, the results were performed in the form of tables and graphs. Chi-square test was performed difference with respect to age. Non-parametric Cochran's Q test was performed to represent the changes in the bacterial counts in both tested groups (*Streptococcus* and *Lactobacillus*). Wilcoxon Signed Rank test was performed to test buffer capacity.

Results

As to gender, no statistically significant difference was found neither with respect to the number of Streptococci and Lactobacilli, or the pH value. With respect to the number of cariogenic bacteria (*Streptococci* and *Lactobacilli*) counted at the 1st visit, before the participants started to use Plidenta Pro-T-action toothpaste, the results obtained after 4 weeks showed a significant reduction in both *Streptococcus mutans* and *Lactobacillus* bacteria counts as rated by CRT tests. The results representing the group with high *Streptococcus mutans* count

($\geq 10^5$) have shown a significant decrease by dropping down from high 78.4% at the 1st visit to significantly lower 26.5% after 4 weeks of using the probiotic toothpaste (Table 1, Figure 1). A decrease in the number of participants with the high *Lactobacillus* count ($\geq 10^5$) was observed as well, significantly dropping down from high 52.9% at the 1st visit to a low 26.5% after a 4 week check-up interval (Table 2, Figure 2). On the contrary, no significant changes were observed with respect to the pH value between a 2nd and 4th week check-up intervals as represented by Wilcoxon Signed Rank test (Table 3) and Figure 3.

TABLE 1
COCHRAN'S Q TEST FOR *STREPTOCOCCUS MUTANS* BEFORE, 2 AND 4 WEEKS AFTER USING TOOTHPASTE

Time interval for check-up	<i>Streptococcus mutans</i> count		Cochran's Q=33.8
	$<10^5$	$\geq 10^5$	
Initial visit (prior to using toothpaste)	10	39	N=49
2 weeks after using toothpaste	23	26	df=2
4 weeks after using toothpaste	36	13	p<0.001

TABLE 2
COCHRAN'S Q TEST FOR *LACTOBACILLUS* COUNT BEFORE, 2 AND 4 WEEKS AFTER USING TOOTHPASTE

Time interval for check-up	<i>Lactobacillus</i> count		Cochran's Q=13.3
	$<10^5$	$\geq 10^5$	
Initial visit (prior to using toothpaste)	23	26	N=49
2 weeks after using toothpaste	32	17	df=2
4 weeks after using toothpaste	36	13	p<0.001

N=total number of participants; df=degrees of freedom

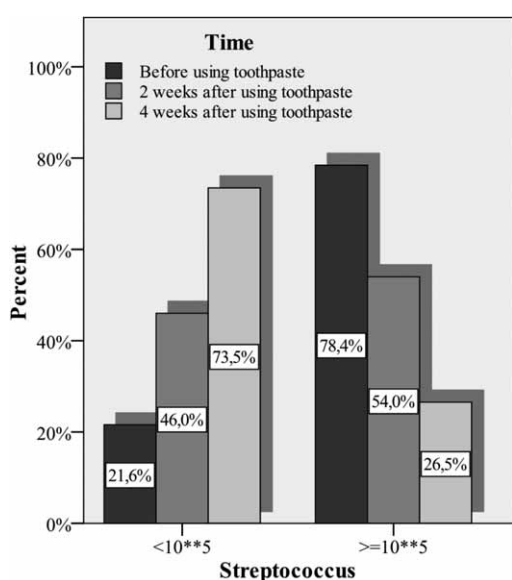


Fig. 1. *Streptococcus mutans* counts at 1st (prior to using pro-t-action toothpaste), 2nd and 4th check-up.

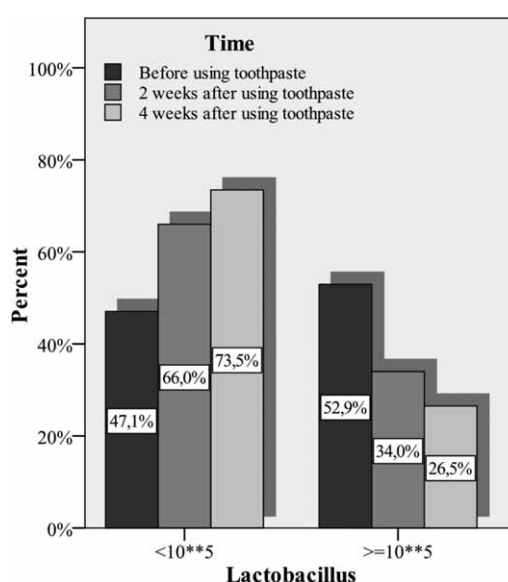


Fig. 2. *Lactobacillus* counts at 1st (prior to using Pro-T-Action toothpaste), 2nd and 4th check-up.

TABLE 3
WILCOXON SIGNED RANKS TEST FOR BUFFER CAPACITY (PH VALUE) AFTER USING TOOTHPASTE

pH value after using toothpaste	N	\bar{X} Rank	Sum of Ranks	Wilcoxon Signed Ranks Test
4 weeks post-treatment <2 weeks post-treatment	9	9.00	81.00	
4 weeks post-treatment >2 weeks post-treatment	8	9.00	72.00	Z=-0.243
4 weeks post-treatment =2 weeks post-treatment	32			p=0.808
Total	49			

N=total number of participants

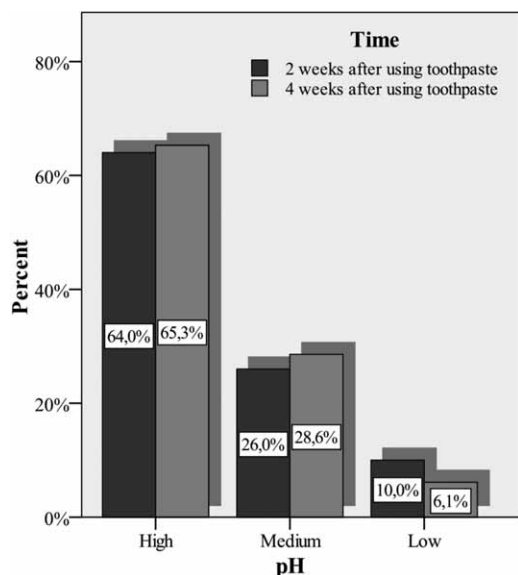


Fig. 3. Buffer capacity (pH values) in participants before, 2 and 4 weeks after using toothpaste.

Discussion and Conclusion

Probiotics include mostly bacteria, but moulds and yeasts as well, and were defined in 2001 as »live microorganisms which confer a health benefit on the host when administered in adequate amounts«^{6,7}. Research studies have so far revealed diverse beneficial effects of probiotics, which potentially range from direct inhibition of pathogenic microbes to improving host immune functions⁸. In this context, lactic acid bacteria have been detected as species which was the most widely used in probiotic preparations. The utmost interest have lately been focused on applying probiotic therapy with the aim of maintaining oral health, i.e. preventing or treating oral health diseases and development of caries, as well as resolving periodontal issues⁹⁻¹¹. Some of the probiotic strains, such as those belonging to the genera *Lactobacillus* and *Bifidobacterium*, have been tested worldwide for their ability to confer probiotic effects on the oral flora. As to dental caries, it is not merely the presence of a single organism, such as *Streptococcus mutans* bacteria, but the interactions between the biofilm residents that appears crucial and determines the properties

of a biofilm^{12,13}. Acid-producing *Streptococcus mutans* and *Lactobacillus* bacteria are believed to play a major role in initiation and development of dental caries¹⁴. After exposed to saccharosis, *Streptococcus mutans* undergoes the process of autoaggregation which results in increasing its number that is further present in saliva. Consequently, these multiplied bacteria adhere to the tooth enamel and being permanently present in the form of oral biofilm are responsible for tooth decay¹⁵. It has been suggested that some probiotic cultures of *Lactobacillus* species can be effective in forming co-aggregations with *Streptococcus mutans* bacteria, which results in further elimination of *Streptococcus mutans* from the oral cavity. This specific coaggregation does not depend on temperature and has proved to be stable in the presence of protease and other proteolytic enzymes or proteins¹⁶. However, co-aggregation is dependant on calcium and cannot be completed in the presence of ethylenediaminetetraacetic acid (EDTA), whereas the presence of saliva ranging from 4.0 to 8.0 pH values does not significantly influence it¹⁷. Furthermore, *Lactobacillus* doesn't seem to show affinity to binding to hydroxyl-apatite crystals or other type of benign oral flora which colonizes tongue, i.e. *Streptococcus salivarius*, which further leads to a conclusion that no opportunistic infections might be expected in the presence of *Lactobacillus species*¹⁷. This innovative approach based on microbiological investigations suggests that some *Lactobacillus* species may exert probiotic effect via different vecchiculum, such as toothpastes or mouthrinses. Our results are in accordance with the proposed presumptions and therefore suggest that the effect of this innovative probiotic toothpaste (Plidenta Pro-T-Action, Neva, Croatia) is due to *Lactobacillus paracasei* contained in the synthesised pro-t-action substance, that has the unique ability to very precisely target the caries causing bacteria and binding exclusively to them further results in clumping them. When aggregated and grouped together, the bacteria can be flushed out of the oral cavity by normal swallowing or rinsing. An advantage of *Lactobacillus paracasei* is that it needs to remain in the oral cavity for only a minimum of ten seconds to become active²³. Some clinical studies have reported up to even 50% decrease in the *Streptococcus mutans* bacteria count after using *Lactobacillus paracasei* mouthrinse¹⁸. A reduction of the amount of *Streptococcus mutans* bacteria in the mouth was reported after using *Lactobacillus paracasei* candies as

well¹⁹. In the light of these recent achievements in science, a new launched toothpaste containing probiotic strain *Lactobacillus paracasei* (Plidenta, Neva, Croatia) represents an innovative product with improved anti-cariogenic properties and has so far shown excellent results in reducing the amount of *Streptococcus mutans* bacteria. The results of this study proved that a daily usage of this probiotic toothpaste interferes with the number of plaque-accumulated *Streptococcus mutans* and *Lactobacillus* bacteria, that have proven cariogenic effects and are regularly present in saliva¹⁹. The results also showed an immediate effect of *Lactobacillus paracasei* on *Streptococcus mutans* and *Lactobacillus* counts which was reported during the 2nd and 4th week intervals of using exclusively the selected toothpaste containing pro-t-action substance. The reported results are in accordance with some microbiological reports which suggested that a certain 2 week-period may be needed for *Lactobacillus paracasei* to become effective in probiotic action²⁰. As opposed to our study, a certain tendency for an increase in *Lactobacillus* counts after consuming some probiotic products contained in foods was suggested according to some clinical study reports^{20–22}. However, some studies on cheese and yoghurt consumption suggested a posttreatment effect which was present for up to

2 weeks after discontinuing the consumption of the products containing probiotic strains of *Lactobacillus* species^{20,23}. It could imply that different *Lactobacillus* species existing in the oral cavity and gastrointestinal tract may exert different probiotic effects. Although probiotics have shown scientifically proven benefits, further studies are mandatory in order to investigate their mechanism of posttreatment action as well. Based on the proposed achievements, researches have been able to use the proposed mechanism of probiotic action giving the emphasis on the metabolism of »good bacteria« that are normally present in saliva. A newly produced toothpaste which contains a laboratory synthesised pro-t-action substance with an active *Lactobacillus paracasei* probiotic strain showed ability to balance *Streptococcus mutans* bacteria and represents a new concept in the oral hygiene measurements. It could be recommended as a therapeutic toothpaste particularly in patients who are at high risk for caries.

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NOVA DOSTIGNUĆA U PREVENTIVNOJ STOMATOLOGIJI TEMELJENA NA ISPITIVANJU NOVE PROBIOTIČKE PASTE ZA ZUBE

SAŽETAK

Umjetno sintetizirani probiotik iz soja laktobacila koji je sadržan u testiranoj zubnoj pasti omogućio je inovativni pristup u preventivnoj stomatologiji. Primjenjen je novi koncept djelovanja mehanizma probiotičkih bakterija, prema kojemu se ekvilibrij bakterijske flore normalno prisutne u usnoj šupljini postiže temeljem mehaničke eliminacije karijesogenih bakterija iz usne šupljine. Ovo kliničko ispitivanje provedeno je u periodu od 4 tjedna na uzorku od 50 ispitanika hrvatske populacije. Cilj istraživanja bio je ispitati učinkovitost zubne paste koja sadrži probiotički soj bakterija, te je kao takva prvi puta proizvedena u Hrvatskoj. U istraživanju su korišteni CRT testovi (Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein) temeljem kojih se individualno u svakog ispitanika procijenio broj karijesogenih bakterija (*Streptococcus mutans* i *Lactobacilla*). Pojedinačni uzorci sline testirani su prije početka korištenja zubne paste, te nakon 2 i 4 tjedna. Ispitanici su nakon detaljnih informacija u vezi s kliničkim testiranjem, potpisali pristanak i, pridržavajući se temeljitih uputstava proizvođača, u narednih 4 tjedna održavali oralnu higijenu koristeći isključivo testiranu zubnu pastu i odgovarajuću četkicu istog proizvođača. Statistički rezultati nakon 4 tjedna korištenja probiotičke zubne paste pokazali su značajno smanjenje broja ispitanika u kojih je na početku istraživanja dijagnosticiran visoki broj karijesogenih bakterija. Broj ispitanika sa visokim brojem streptokoka značajno se smanjio sa 78,4 na 26,5%, a broj ispitanika sa visokim brojem laktobacila značajno je pao sa 52,9 na 26,5%. Rezultati istraživanja ukazuju na značajnu učinkovitost testirane zubne paste, a koja se potencijalno može pripisati djelovanju sintetički proizvedene probiotičke supstance koja je sadržana u testiranoj zubnoj pasti. Stoga ovo kliničko istraživanje otvara nove mogućnosti glede inovativnih tehnologija temeljem kojih se probiotici koriste u svrhu održavanja ravnoteže bakterijske flore, poglavito u skupinama visokog rizika i sklonosti spram karijesa.